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EXPERIMENTAL MEDICINE

A TERATOMA OF THE ABDOMINAL CAVITY.

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PLATES XIX–XXII.

Teratomata have always attracted attention by their strange appearance, but of late years they have given rise to much investigation on account of the promise they hold out of elucidating the etiology of the true tumors.

The tumor to be here described was a solid teratoma of the abdominal cavity, in itself a sufficiently rare occurrence, and, besides, was remarkable on account of the great variety of tissues which it contained. Clinically also it took an unusual course, for, after being operated on and apparently entirely extirpated, it rapidly recurred.

In May, 1893, Dr. W. F. McNutt was called by Dr. J. S. Stone to see a patient, a girl, 12 years of age, who was suffering from an abdominal tumor. The patient herself had noticed ten months previously that she was growing stouter and that her clothes no longer fitted her. The rate of growth had at first evidently been slow, but latterly very rapid, as Dr. Stone for a few days previous to the consultation had been able to make out an increase in girth of one and one-half inches a day. Both previous to and after the enlargement of the abdomen the patient had always enjoyed good health. There was no loss of flesh and she had never suffered any pain. There was nothing in the family history to

indicate any hereditary inclination to the formation of neoplasms. One grandfather had died of tuberculosis. There was no history of multiple pregnancies on either side of the house.

On physical examination the abdominal veins were found dilated and the abdomen was considerably enlarged. The swelling was general, and much of it was evidently due to ascites. By palpation and percussion a solid mass could be made out in the right side of the abdomen.

On May 27, 1893, the abdomen was opened, and the tumor, together with about two gallons of slightly bloody ascitic fluid, was removed.

When Dr. McNutt brought me the specimen he said that the tumor was found on the right side of the abdomen and in the peritoneal cavity; that its general shape, as it lay in the abdomen, was long, narrow and curved, and that the shorter arm of the curve lapped around the caput coli, while the longer arm extended up along, and was attached to, the outer side of the ascending colon throughout nearly its whole length to a point nearly as high as the liver; that it shelled out easily, and when it was taken away the operation seemed to be complete, as there was no appearance of any other diseased tissue in the abdominal cavity. Dr. McNutt also told me that in removing the tumor no large blood-vessels were encountered, the vessels being only such as run in brittle adhesions; and while that part of the peritoneum from which the growth was detached was of course raw and bleeding, the rest of the peritoneal surface, as far as could be seen, was perfectly smooth, glistening, and normal. After the operation the abdominal cavity was closed and the patient made a speedy and uneventful recovery for the time being.

A photograph of the tumor is shown in Plate XIX, Fig. 1. When I first saw the original tumor, a few hours after the operation, it lay in a basin, a formless, quivering, semi-fluid, jelly-like, shiny mass, with white, greasy patches on its surface, and small pieces of bone, the size of a silver half-dollar and less, scattered in its substance. After being in alcohol for some time the tumor became firmer and assumed more definite outlines. It weighed two pounds, and resembled very strikingly a cerebral hemisphere. Like this it was elongated and lobulated, and all the lobules were smooth and grew out in one direction as if they sprang from a common elongated base. Two of the fissures cut into the tumor so deeply that they divided it into three parts, a large central portion with a smaller portion at each end. These three lobes, besides being united at their bases, were con-

nected together with strings of tissue thrown across the fissures. On cutting into the tumor it was soft, and in many places honeycombed with minute cysts, many of which were so large that they could be seen with the naked eye. The largest cyst was about the size of a cherry; then there were a number of pea-sized cysts, but by far the greatest number were very minute and could only be made out with a lens or a microscope. In spite of this cystic structure, however, the tumor, looked at as a mass, was a solid growth, a point of a good deal of importance considering the rarity of solid dermoids in the interior of the body.

The term solid teratoma or dermoid does not mean that the tumor does not contain cysts. In fact such teratomata contain large numbers of cysts, but these cysts do not interfere markedly with the general solid appearance of the neoplasm, while in the so-called cystic neoplasms of this class the hollow character of the tumor is the main feature.

Almost the whole of the outer surface of the tumor was covered with a distinct epithelial membrane. In many places this covering was clearly skin (Plate XIX, Fig. 2), being provided with fine downy hair and sebaceous glands. In other situations it was devoid of hair and looked more like the mucous membranes that are covered with flat, stratified epithelium. This epithelial covering, provided or unprovided with hair, followed the irregularities of the tumor down into the deepest fissures. On some parts of the free surface evidences of an epithelial covering were not distinct to the naked eye, but even in such places on microscopic examination there could be made out occasional indications of a papillary layer, or groups of cells indicating probably the remains of an epithelial covering.

In most dermoids the skin lines one or more of the cysts of which the tumor is composed; in this tumor, however, nearly all the cutaneous integument was found on its external surface, and only one small cyst was discovered lined by skin.

The extent of this cutaneous envelope, its position on the outside of the tumor, the ease and apparent completeness with which the tumor was shelled out of the peritoneal cavity, were noteworthy facts,

making it seem probable that this teratoma was an independent individual enclosed, to at least a great extent, in its own skin.

Although a number of small pieces of bone were present in the tumor, none of them resembled at all clearly in shape any definite human bone. Nor did the islands of cartilage here and there met with (Plate XX, Fig. 4) show a form recognizable as that of any of the cartilages or bones of the body. There was nothing in the tumor therefore which could justly be called a skeleton, nor could it be made out, as is sometimes possible in teratomata, that the bones were arranged according to the conformation of the fœtal skull. But, as will be shown further on, one of the pieces of bone may have represented a fusion of some of the bones in the neighborhood of the ear. All the pieces of bone, as well as the islands of cartilage, were bound in the tumor by strong attachments, so that it was difficult to free them from the surrounding tissues. The largest piece of bone had a hollow in it enclosing an empty tube about half an inch in diameter. The total weight of the pieces of bone amounted to about one ounce. No teeth were found anywhere in the growth.

As has been said, in many places the tumor was honeycombed and spongy with minute cysts, but in other regions there were very few cysts, and in still other areas of considerable size there were none at all. Some of the cysts communicated with one another. The majority were filled with a mucoid substance, and none of them were filled with sebum. No polypoid or warty projections were found jutting into the cysts. Some of the cysts had a refractive yellowish lining as smooth as peritoneum, and one of them was found lined with skin.

The appearance of the tissue in among the cysts and between the bones and masses of cartilage varied considerably. In some places it was fairly dense and tough and apparently fibrous; in others, much softer and even jelly-like. In many parts of the stroma there were streaks of black or brownish-black pigment, and in one place a sheet of tissue, measuring 1 x 2 cm., covered on one side by a thin, continuous, even, black pigment layer, was found attached by one of its edges to a bone in the centre of a large lobe. The other relationships

of this sheet of tissue could not be determined owing to the disturbance of the parts in getting out the bone. This sheet of tissue is interesting because, on microscopic examination, it was shown to be a part of an eye.

Pieces of the tumor, hardened in alcohol, were embedded in celloidin, sectioned and stained by various methods. A study of the finer structure of the growth revealed even more complicated relations than the gross appearances had indicated. The growth contained tissues and portions of organs corresponding in embryonic origin to all three of the germinal layers. Corresponding to the epiblast there were skin with cutaneous organs and appendages, central nervous system, peripheral nerves, and rudiments of eye structures. The hypoblast was represented by mucous glands, tubes and cysts with epithelial lining and surrounded by smooth muscle. The mesoblastic tissues consisted of bone, cartilage, white fibrous tissue, yellow elastic tissue, mucoid connective tissue, adipose tissue, smooth muscle fibre, and blood-vessels.

The best developed organ in the teratoma was the skin with its hairs and attendant sebaceous glands. This is a regular phenomenon in such tumors, and it is usually such a prominent feature as to give them the name dermoid. This predominant participation of the skin is supposed to be due to the fact that the ectoderm has the start of the other fœtal layers in development. The skin in the case under consideration was so far advanced that there was at any rate a commencement of defluvium of the hair (Plate XX, Fig. 6). The embryonic nervous system, the eye-structures, and structures belonging to the intestine were also remarkably developed. Microscopic examination of the skin of the surface of the tumor showed the presence of nearly all the constituents of normal skin—epidermis, corium, tela subcutanea, hairs with hair follicles, sebaceous glands, and blood-vessels. In one section of skin a definite bundle of mature medullated nerve fibres was found. As so frequently happens in dermoids, no sweat glands were discovered.

According to Wilms,* the presence of tissue of the central nervous

* Wilms, Ueber die Dermoidcysten und Teratome, mit besonderer Berücksichtigung der Dermoide der Ovarien, *Deutsch. Arch. f. klin. Medicin*, lv, 289.

system in dermoids seems to be a common occurrence, for of nineteen consecutive cases of dermoid cyst of the ovary examined by him he found such tissue in eighteen. Professor W. H. Welch first drew my attention to the presence of tissue of the central nervous system in this tumor, and its description will be left to Dr. Lewellys F. Barker (p. 284 et seq.).

It has been previously mentioned that a sheet of tissue was found covered by a thin, continuous, even, black layer, and attached by one of its edges to a bone in the centre of a large lobe. Transverse sections of this sheet of tissue showed the black layer to correspond to the pigment epithelium of the retina. There were flat polygonal cells filled, excepting the nucleus, with dark brown pigment, and these cells were so placed as to form a mosaic (Plate XXII, Fig. 11). This epithelium lay on a slightly pigmented connective tissue that was much firmer than the soft connective tissue usually encountered in this tumor. No definite tunica vasculosa (chorioidea) could be made out. The pigment epithelium could have been nothing but the lamina pigmentosa retinæ, as there is no other structure in the human body that resembles it; and the firm, slightly pigmented, in places distinctly laminated connective tissue on which it lay was probably the sclera.

Structures corresponding evidently to fœtal intestine were found. This could be seen as an oval tube cut transversely and lined with exquisite cuboidal epithelium. Numerous villus-like processes projected into the lumen. Beneath the epithelium there was a layer of cellular connective tissue, and the whole tube was surrounded by a definite tunica muscularis consisting of bundles of smooth muscle fibres, cut chiefly obliquely, but in part transversely (Plate XXI, Fig. 8).

Another tube lined with cylindrical epithelium lay close to a small mass of cartilage. This might have been embryonic trachea, though no smooth muscle could be made out in its wall, nor did the lining epithelium bear demonstrable cilia. Cysts with mucus in the lumen and lined by ciliated columnar epithelium with beautiful goblet cells were, however, also found, and doubtless corresponded to some part

of the respiratory tract. Nothing recognizable as liver or pancreas was found.

The tube, which has already been mentioned as having been removed from a concavity in the largest piece of bone, and which was about 1 cm. long and 7.5 cm. in diameter, was examined microscopically. The wall of the tube contained a great deal of elastic tissue, the delicate fibrils of which were well brought out by the orcein stain. In some places the elastic tissue was arranged in the form of alveoli containing cartilage cells. There were also numerous large hairs in the wall of the tube. This tissue was therefore elastic cartilage, and as it was a tube situated in the hollow of a bone, and as there were also large hairs in the wall of the tube, this elastic cartilage may have been from the external ear. It is also of interest that this hollow bone with its tube containing elastic cartilage was situated near the sheet of tissue containing the flat pigmented epithelium of the eye already described. The bone was too large for the quadrate bone, but it may have represented a fusing together of several bones in the neighborhood.

The stroma of the tumor, made up of mesodermal structures, was not inferior to the epithelial elements in point of variety. There were little masses of hyaline cartilage showing concentric arrangement of cells toward the periphery, and each mass was surrounded by a well marked perichondrium; bone with bone corpuscles and canaliculi; mucoid connective tissue; white fibrous tissue; yellow elastic tissue; adipose tissue; embryonic connective tissue, resembling round-celled and spindle-celled sarcomatous tissue; and long bands of smooth muscle fibre. Then there was a great deal of intracellular and diffuse (intercellular) dark brown, granular pigment; and the tumor was well supplied with small blood-vessels, arteries, capillaries, and veins, having for the most part embryonic walls. No trace of a heart was found. The blood in the blood-vessels had all the characteristics of adult blood. No nucleated red blood corpuscles were seen. The white and red blood corpuscles appeared to be present in normal proportions.

No striated muscle fibre was found in the tumor. All the structures in the interior of the tumor were shuffled up together without

any ascertainable law or order. There was an immense variety in the kind of tissue lining the cysts; some, as said, were clothed with flat, stratified epithelium; others with simple columnar epithelium; others with stratified columnar epithelium; others with ciliated columnar epithelium; and still others with goblet cells (Plate XX, Fig. 3). There were also many irregular cysts or alveoli containing stratified columnar epithelium forming wild-looking figures and broken festoons scattered about in a disorderly fashion in the alveoli, and without a basement membrane, as in adeno-carcinoma.

The cyst situated near the choroidal structure, before mentioned, was lined with skin provided with hair and sebaceous glands. This was the only unequivocal dermoid cyst within the tumor, as all the rest of the skin was on the outside of the tumor instead of lining a cyst. There were other cysts lined with flat stratified epithelium, but this, of course, might have been mucous membrane and not skin.

In many places there were masses and strings of round or polyhedral epithelial cells forcing their way between the fibres of the stroma of the tumor as in ordinary carcinoma (Plate XX, Fig. 7). There was also the formation of epithelial pearls and of onion-like bodies, which so frequently happens when flat epithelium grows in a confined space, as notably in flat-celled epithelioma.

Everything went well after the first operation for a few weeks, and the child appeared to enjoy her usual good health, but in July, a little over a month after the first operation, the abdomen was seen to be enlarging again.

A second operation was performed July 15, 1893, when a mass about as large as that removed at the first operation was taken away. At the first operation the tumor shelled out easily and the operation appeared to be complete. The second operation, however, Dr. McNutt told me, was manifestly incomplete. The friable, glairy, recurrent tumor, which was located mainly on the right side of the peritoneal cavity, had to be removed in pieces and could not be enucleated, and much of the diseased tissue could not be removed at all, as it had spread out laterally and involved a much larger surface of

the peritoneum. At this operation a considerable quantity of bloody serum was found in the abdominal cavity, but not nearly so much as at the first operation. The abdomen was closed, and the patient died July 18, 1893, 51 days after the first operation.

After death Dr. McNutt made a simple incision into the abdomen and found that the diseased tissue had spread as a continuous layer of glairy, friable tissue over the visceral peritoneum of the lower part of the abdomen behind the bladder and nearly across to the left side of the abdominal cavity. The parietal peritoneum showed no evidence of disease. There did not seem to be any involvement of the intestinal wall excepting the growth of the neoplasm in the peritoneum covering the intestine. The abdomen only was opened and none of the viscera were cut into; the liver, the spleen, the kidneys, the uterus and ovaries were not incised, but they all appeared to be normal as far as could be judged from the external surface. The thoracic cavity was not examined. Very little fluid was found after death in the abdominal cavity.

The quick recurrence of the tumor *in loco*, its rapid growth, its spread by lateral extension, the appearance of the soft, glairy, brittle tissue removed at both operations, as well as the occurrence of bloody fluid in the peritoneal cavity in both instances, were enough to establish the identity of the recurrent growth and the original tumor, and the microscopical examination was confirmatory of this in every respect.

The general character of the recurrent growth, like the original, was that of a solid tumor richly studded with cysts, most of which were microscopic in size. A great many of the cysts were, however, as large as a pea, and some of them of the size of a walnut. As in the original tumor there was a great variety in the kind of tissue lining the cysts, and one cyst was sometimes clothed with many varieties of epithelium; for instance, columnar epithelium with nuclei far from the base of the cell was continuous with columnar epithelium with nuclei near the centre or near the base. This in turn was continuous with ciliated epithelium, which was finally continuous with round or polyhedral epithelial cells. As another example, an irregular cyst

was lined on one side with stratified columnar epithelium, and the opposite side was covered with stratified flat epithelium, while a great number of flat epithelial cells were desquamated into this cyst cavity. In another cyst, stratified columnar epithelium tapered off into a single layer of cuboidal epithelium. There was a great number of well formed tubes lined entirely with stratified columnar epithelium. Many cysts were lined either partly or entirely with a single layer of beautiful columnar epithelium, each cell of which had a single nucleus placed far out at its distal extremity. Examples of this kind of epithelium were found both in the original and in the recurrent tumor, and were at first thought to represent the membrana adamantina of the fœtus, but a hair was discovered in the original tumor, in which the outermost cells of the external root sheath had exactly these characteristics; the cells formed very long columns, much longer than they usually do, and each cell was provided with a single nucleus that lay far out at its distal extremity (Plate XX, Fig. 5). Therefore, although the cells that looked like those of the membrana adamantina might really have been so, yet there was a possibility that they might have belonged to hair follicles where the development stopped with the formation of the external layer of the external root sheath. In further support of the pilary origin of these cells many follicles were detected having about the diameter of a hair follicle, and lined with a single layer of columnar cells, with their nuclei far out toward the free extremity of the cell.

The recurrent growth, like the original, contained tissues from all three of the germinal layers. Indeed nearly all the structures met with in the original tumor could be found in various parts of the tissue removed at the second operation. There were large masses of tissue of the central nervous system. The endodermal structures were scattered through the other tissues in considerable amounts, for instance, cysts lined with ciliated and columnar epithelium, and spaces lined with goblet cells.

The mesodermal stroma of the recurrent tumor was similar to that of the original, and consisted of little globes of hyaline cartilage surrounded by perichondrium, mucoid connective tissue, adipose tissue,

well formed fibrous tissue, embryonic round-celled and spindle-celled connective tissue resembling sarcomatous tissue. One small flat piece of bone was removed with the tissue taken away at the second operation, but of this more hereafter.

In the immediate neighborhood of some of the tubes lined with lamellated epithelium, stratified columnar epithelium could be seen to form the wildest wreaths and broken festoons, scattered in irregular cavities much as in adeno-carcinoma. There were also places where masses of round epithelial cells could be seen invading the stroma of the tumor and pushing along between its fibres as in ordinary carcinoma.. There was one particularly striking example of the metaplasia of columnar into flat epithelium (Plate XXI, Fig. 9). This was found where several follicles lined with simple columnar epithelium appeared to meet. The epithelial walls of two of these follicles were imperfect, and the epithelium at these imperfect points, as well as that lining the mouth of a third follicle, seemed clearly to have changed into flat epithelium with pearl formation. Of course there was a possibility that an invasion of flat epithelium might have taken place, but any one looking at the specimen would be surely drawn to the view that it was an instance of metaplasia of columnar to flat epithelium. Nests of flat epithelium containing pearls were found in the recurrent as well as in the original tumor (Plate XXI, Fig. 10).

That this tumor was a teratoma there can be no doubt, as is shown by the variety of tissues entering into its structure. It being settled that the tumor was a teratoma, the determination of the exact locality in which it grew is the next point to be considered.

As mentioned by Ernst Schreiber,* teratomata are oftenest found in the region of the head or sacrum. The next most frequent locality for their growth is the genital glands, and therefore the possibility of the tumor under discussion having originated in the ovaries must be carefully considered. But decidedly against this view is the fact that the growth lay high up in the abdominal cavity, and well away from the ovaries, which were found perfectly healthy and in their

* Virchow's *Archiv*, cxxxiii, 165.

usual situation. Furthermore, no strands of tissue binding the tumor to the ovaries were encountered during the operation for removal.

Another remote possibility presents itself, that, as in Kolaczek's case,* tissues from an ovarian dermoid might have been transplanted from the ovary to the peritoneal cavity and have grown there; but in such an event the original dermoid or teratoma would have been quite obvious in the affected ovary, while in the case under consideration the ovaries were expressly stated to have been found healthy.

According to Wilms,† very few instances of dermoid have been reported as occurring in the abdominal cavity proper, excluding those of the pelvis, most of which were single cysts, and judging from the cases reported in the literature instances of solid abdominal teratoma must be exceedingly rare. But Meckel, Bonfigli, Marchand and Maydl have each reported abdominal growths of a more complicated structure than simple dermoid cysts. Meckel's ‡ tumor was situated on the diaphragm and contained twenty-one pieces of bone, four teeth and some hair. Bonfigli's § case was that of a pear-shaped growth occurring in a strand of tissue between the liver and the stomach. It contained a piece of true bone in which were found two teeth, and nineteen more teeth lay free in the tumor cavity. The main cyst was lined partly with cutis. There were other cysts filled with mucus, but they were not further described.

One of the most complicated teratomas of the main abdominal cavity was described by Marchand.|| It was situated behind the peritoneum, and on the aorta and left kidney, and contained a skull cavity, intestine, male genitals, prostate and cysts lined with ciliated epithelium. This case was therefore very far advanced developmentally, and was analogous to the following reported by K. Maydl¶: In a well developed but very anæmic man, 19 years of age, a tumor

* Virchow's *Archiv*, lxxv, 39.

† Loc. cit.

‡ *Deutsch. Arch. f. Physiol.* (1815). Quoted by Wilms.

§ Ref. Schmidt's *Jahrb.* clxx (1876), 180. Quoted by Wilms.

|| *Breslauer ärztl. Ztschr.*, 1881, No. 21. Quoted by Wilms.

¶ *Wiener klin. Rundschau*, x (1896), 295. Abstract in *Archives of Pediatrics*, December, 1896.

was found occupying the greater part of the pelvic cavity. Laparotomy was performed and the tumor was seen to lie between two folds of the root of the mesentery, and consisted of a well formed trunk and limbs. The head was absent, and in its place there was a tuft of hair 50 cm. long. The fœtus was enclosed in a firm amniotic sac that contained also some oily fluid. A thickening of the amniotic membrane, connected with the superior mesenteric artery, showed histologically the structure of the placenta. The umbilical cord was entirely wanting. The tumor was classed as a dermoid and was assumed to be a rudimentary twin, an instance of *fœtus in fœtu*.

My case evidently is to be placed in the same category with the four cases just mentioned, and it is almost unnecessary to draw attention to the parallelism between the two last mentioned tumors, Marchand's and Maydl's, and the growth we are now considering. All three have made the impression on those examining them that they were dealing with tumors that were in reality independent individuals. All three were situated in the abdominal cavity; two of them, Marchand's and Maydl's, retroperitoneally, and one of them, the case in hand, intraperitoneally.

The age at which teratomata and dermoid cysts develop appears to vary much. Wilms, in his article previously referred to, speaking more particularly of dermoids of the ovary, makes the statement that they may appear at any time of life between intrauterine existence and the seventieth year, although the number occurring in childhood and old age is small, as the majority of cases occur during the second and third decade. In the case here reported the patient was twelve years of age. As the child had not yet begun to menstruate, no connection between a physiological increase of blood supply to the abdominal organs and the growth of the tumor could be definitely proven.

The relatively great development of the ectodermic structures (integument and nervous system) in the tumor is quite in accord with other knowledge, for we have evidence that both ontogenetically and phylogenetically the ectoderm takes precedence of both the endoderm and mesoderm. In speaking of dermoids of the ovary, Wilms says that "the advanced development of the ectoderm as well as the pre-

ponderance of the tissues of the head in these tumors are easily explained by the early differentiation, both of the epiblast and of the cephalic extremity in the fœtus." Wiedersheim,* in referring to the relative age of the ectoderm, states that the dermal skeleton is phylogenetically older than the endo-skeleton. Its relative age is shown not only by palæontology but also by ontogeny, *e. g.* the young file fish is provided with a complete dermal armor at the time when the ossification of the primordial cranium has hardly begun.

Our case is somewhat remarkable in that it is the third instance on record where the hair of a dermoid has been found growing free into the abdominal cavity. The first case is that reported by Kolaczek,† in which the patient, who was a spinster, 45 years of age, was operated on for a dermoid cyst of the ovary. At the operation a number of lentil-sized yellowish nodules were found scattered over the visceral and parietal peritoneum. Many of these nodules were pierced through their centre by a fine hair, and were regarded by Kolaczek as miliary metastases from the dermoid of the ovary. According to Wilms, Fraenkel ‡ has reported a similar case.

Until recently the existence of nervous tissues in dermoids and teratoma has been considered to be rare. Baumgarten,§ writing in 1887, made the statement that only six authenticated observations of the finding of central nervous tissues preceded that of his case. Recently, however, it has been shown that such tissue is really of extremely common occurrence, and one must believe that its supposed rarity in the earlier cases is due to the fact that it was not recognized. Wilms believes that tissues of the central nervous system are almost always, if indeed not constantly, present within ovarian dermoids, for he found it in 18 out of 19 consecutive cases which he examined. He believes furthermore that the finding of such tissue in a teratoma is a good argument for considering the particular tumor in which it is found as an independent individual.

* Elements of the Comparative Anatomy of Vertebrates, p. 30. London, 1886.

† Loc cit.

‡ *Wien. med. Wochensch.*, 1883.

§ Virchow's *Archiv*, cvii, 515.

Wilms, in his investigations, frequently ran across corpora amylacea, and he states that they were of great value to him in locating the tissue of the central nervous system. In the very numerous sections of our tumor examined no corpora amylacea were found.

Eye structures have not been encountered very frequently, as up to the present I have been able to collect from the literature records of only fifteen cases where pigmented epithelium has been demonstrated in teratomata and dermoids. These cases have been reported by Marchand,* Baumgarten,† Lazarus,‡ Wilms § (3 cases), Kappeler,‖ Pommer,¶ Rippmann,** Verneuil,** Kümmel,** Benno and Martin Schmidt,** O. Spöndly,** van Duyse,†† and Lovett and Councilman.‡‡ If the case in hand is added to the above mentioned we have a total of sixteen where pigment or pigmented epithelium has been found in teratomata.

The question of the recurrence of the growth and its malignancy has next to be considered. It has seemed to me worth while in this connection, on account of the great interest of this side of the subject, to review with some care the cases of teratoma which have been reported as recurrent or malignant.

The instances where teratomata have been found, either clinically or microscopically, to be malignant, or to have undergone malignant degeneration, are not numerous. This is somewhat surprising when one reflects that teratomata have been considered to be misplacements of fœtal tissue, and that true tumors, the malignant ones included, are also, according to Cohnheim's theory, supposed to originate from misplacements or implantations of fœtal tissue. The experiment of engrafting fœtal tissue into animals with a view to causing tumor formation has been tried fruitlessly, but, as Grawitz has said, teratomata fur-

* Loc. cit.
† Loc. cit.
‡ Inaug.-Diss., Giessen, 1888.
§ Loc. cit. and Ziegler's *Beiträge*, xix.
‖ Inaug.-Diss., Zurich, 1896.
¶ Abst. in *Centralbl. f. allg. Path. u. path. Anat.*, i (1890), 260.
** Cited by Kappeler, loc. cit.
†† Ref. Schmidt's *Jahrb.*, ccxlix (1896), 235.
‡‡ *Journal of Experimental Medicine*, ii (1897), 427.

nish an example of the implantation of fœtal tissue better than any artificial implantation could, for in teratomata the implantation of fœtal tissue occurs in fœtal tissue, while artificially the grafting must be done at least on post-gestational tissue. The instances I have collected in which teratomas have been malignant, or have undergone malignant change, are as follows:

I.—Czerny's case.* A congenital sacral tumor, consisting for the most part of fat, and containing cysts lined in some instances with ciliated, in others with flat, epithelium. The patient was 55 years of age, and the tumor underwent malignant change after repeated traumatisms. Czerny demonstrated the metaplasia of ciliated into flat epithelium, with pearl formation. After extirpation there was local recurrence, followed by metastasis of flat epithelial cancer in the inguinal glands.

II.—Virchow's case.† Teratoma myomatodes mediastini. This was a large, lobulated, and, for the most part, smooth tumor occupying the right side of the chest of a man, 22 years of age. The tumor contained striated muscle tissue, sarcomatous tissue, and masses of hyaline cartilage, surrounded by well marked perichondrium. There was a considerable number of cysts in the tumor, some of which resembled proliferating ovarian cysts. Other cysts were lined with skin, others with columnar ciliated epithelium, and still others with simple columnar epithelium. In the middle, more compact mass of the tumor there was tissue having the structure of carcinoma. A metastatic nodule in the left third rib was cystic (simple cysts), sarcomatous and carcinomatous, and contained striated muscle fibres, and a portion of this metastasis recalled vividly the structure of fœtal lung. The structure of nodules in the liver and kidneys was not determined.

III.—Jores' case.‡ Combination of an intrathoracic dermoid cyst with malignant cystosarcoma of the left lung. There was nothing of striking interest in the dermoid cyst itself. Its walls, however, were cystic, and these cysts evidently arose from the sebaceous and sweat glands of the dermoid. The intercystic stroma contained many islands of hyaline cartilage, much smooth muscle fibre, and much spindle-celled tissue. Besides the above mentioned cysts there were others more irregular in shape and lined with cylindrical epithelium. This cylindrical epithelium did not sit on a basement membrane, but sat directly on the

* *Arch. f. klin. Chir.*, x, 894.

† Virchow's *Archiv*, liii, 444.

‡ Virchow's *Archiv*, cxxxiii, 66.

spindle-celled tissue. The metastatic nodules in the right lung had exactly the same structure as the main tumor.

IV.—Virchow and Litten's case.* A case of androgyny with malignant teratoid cystoma of the right ovary and bilateral cystic hydrocele of the processus vaginalis of the peritoneum. A multilocular cystic tumor, apparently of the right ovary, developed in a female, 16 years of age. Here and there in the tumor were small dermoid cysts. Medullated nerve fibres, smooth muscle fibres, and masses of hyaline cartilage provided with perichondrium were present in the stroma. In some places the tumor was sarcomatous (round-celled) and in others myxomatous.

In the metastatic nodules in the liver there was neither epidermis nor hair nor cartilage. There were small and large cysts, like ovarian cysts, and the stroma corresponded to the sarcomatous and myxomatous portions of the original tumor. No carcinomatous structure was seen.

V.—Wernitz's case.† A teratoma of the right ovary occurring in a woman, 25 years of age. The tumor had a very complicated structure. The main part of it was formed of small colloid and dermoid cysts. Some of the cysts were lined with simple epithelium, others with columnar epithelium. The dermoid cysts were lined with stratified horny epithelium, and provided with hair and sebaceous and sweat glands. In many places in the connective tissue stroma there were hyaline cartilage and small lamellæ of bone. In other places there was large-celled sarcomatous tissue, and here and there were groups of epithelial nests.

At the autopsy metastatic nodules were found in the peritoneum, lungs, kidneys and liver. These metastatic nodules consisted of richly cellular sarcomatous tissue that resembled closely that of the primary tumor.

VI.—Keller and Kramer's case.‡ The patient was a multipara, 20 years of age, who suffered from a quickly growing, nodular tumor of the right ovary. There was much ascites. The tumor was surrounded by a hard fibrous capsule, and scattered throughout it were many small islands of cartilage and pieces of bone. There were some cysts, one the size of a fist, but the tumor was for the most part solid. Microscopically there were sarcomatous tissue, fat, cartilage and bone. Some of the cysts were lined with stratified epithelium, and others with stratified epithelium that gradually changed into cylindrical epithelium. These cysts were provided with sweat and sebaceous glands and hair. Other cysts were lined entirely with simple or stratified epithelium and had no

* Virchow's *Archiv*, lxxv, 329.

† *Ztschr. f. Geburtsh. u. Gynak.*, xxxi, 417.

‡ *Ztschr. f. Geburtsh. u. Gynak.*, xii, 170, and xviii, 124.

sweat glands, etc. Smooth muscle fibre was present, but no striated muscle fibre and no nerve tissue.

After the operation there was a quick return of the ascites and a recurrence of the tumor.

The patient died eleven months after the operation, and the post-mortem examination showed an extensive return of the tumor in the peritoneum and metastases in both lungs. Microscopical examination showed the secondary tumors to be sarcomatous, and in one of them, situated in Douglas' sac, islands of cartilage were found.

VII.—Emanuel's case.* A girl, 15 years of age, was operated upon, January 30, 1892, for a tumor of the left ovary, the size of a man's head. A quantity of brown ascitic fluid was found in the peritoneal cavity. There were a great number of small cysts scattered throughout the tumor, some lined with skin provided with hairs and sebaceous glands, others had no lining membrane, others were clothed with stratified cylindrical epithelium, and others with simple cylindrical epithelium. Some cysts were lined with cylindrical epithelium that gradually changed to cuboidal or flat epithelium. One cyst was lined with ciliated stratified epithelium. The mass of the tumor consisted of round-celled sarcomatous tissue divided off into fields by strands of connective tissue. There were also smooth muscle cells, small oval or round masses of hyaline cartilage provided with perichondrium, fat, mucoid tissue, and black and golden-yellow pigment partly intra- and partly intercellular.

The recurrence was speedy, for in five weeks there was a demonstrable ascites, and in a fortnight more there were unequivocal symptoms of the presence of an abdominal tumor. The patient died, June 2, 1892, four months after the operation.

There was a recurrence *in loco*, and the right ovary, that at the time of operation seemed to be perfectly healthy, was now changed into a tumor the size of two fists. There were metastatic tumors in the subcutaneous cellular tissue of the abdomen, on the parietal and visceral peritoneum, on the under surface of the diaphragm, in both omenta, the suspensory ligament of the liver, and on the lower surface of the liver. Microscopically, the recurrence *in loco* and the tumors of the omenta were identical with the original growth. The tumor of the right ovary was also similar, but with the addition of blood cysts. The tumors of the peritoneum resembled the sarcomatous stroma of the original neoplasm. The tumor lying in the subcutaneous cellular tissue of the skin of the abdomen was a surprising structure, and is of the utmost interest

* *Ztschr. f. Geburtsh. u. Gynäk.*, xxv, 187.

to us, as it was not simply a sarcoma, but a compound tumor like the original neoplasm of the left ovary. It had a complete connective tissue capsule. The stroma of the tumor was, as in the original, composed of round and spindle-celled sarcomatous tissue, and there were numerous cysts, lined with various kinds of epithelium, some with simple or stratified columnar, others with flat or cuboidal epithelium. As in the original tumor, there were solid columns of flat epithelial cells, and some of the cysts lined with flat epithelium did not have a basement membrane, while all the other cysts had. Besides these structures small pieces of cartilage and sections of glands were found.

VIII.—Lazarus' case.* A tumor weighing 9000 grammes was removed from the right ovary of a woman, 27 years of age. It was decidedly lobulated; the centre of each lobe was formed of cartilage, and around the cartilage the tissue was honeycombed with small cysts.

The following tissues were found on microscopical examination: Hyaline cartilage merging into fibro-cartilage and mucoid tissue; connective tissue, some of which was sarcomatous; adipose tissue; myxomatous tissue with branched cells; elastic tissue; flat epithelium with cholesteatoma pearls; cysts with distinct flat epithelium; cysts formed from dilated glands; and cysts lined with skin provided with sweat glands, hair follicles, but without hair or sebaceous glands. In addition to these structures there were smooth and striated muscle fibres; glands with simple cylindrical epithelium; typical goblet cells like those of the intestine; cysts with ciliated epithelium; neuroglia; varicose medullated nerve fibres; ganglion cells like those of the brain and sympathetic system; and finally eye-like structures. These last lay in the neighborhood of tissue resembling gray brain-matter.

No hair nor sebaceous glands nor bones nor teeth were found. The author, however, believed he discovered indications of fœtal tooth formation.

The tumor grew with immense rapidity, and other tumors were found in Douglas' sac and on the diaphragm which were thought to be implantation growths. The growths in Douglas' sac contained cartilage, and cysts lined with goblet and cylindrical epithelium; those on the diaphragm were made up entirely of mucous tissue.

IX.—Biermann's case.† This was a spindle-celled sarcoma of the ovary, the size of a man's head, and contained a dermoid cyst the size of an apple.

* Ein grosses Teratom des Ovarium. Inaug.-Diss., Giessen, 1888. Abstract by Wilms in Ziegler's *Beiträge*, xix, 369.

† *Prag. med. Wochensch.*, 1885, No. 21.

X.—Ewald's case.* From a girl, 21 years of age, a teratoma of the ovary the size of a man's head was removed by laparotomy. Microscopical examination did not reveal any carcinomatous or sarcomatous tissue. Three weeks later there was a recurrence as large as the first tumor; this was removed, and at the same time the opposite ovary was excised, as it was also the seat of teratoid disease. A further recurrence was demonstrable ten days after the second operation, and two weeks later the recurrent tumor had attained to the size of a child's head. After several weeks a third operation was done, but the patient died. The autopsy showed that the tumor in the last instance had arisen, not from the region of the ovary, but from the omentum; the peritoneum was studded over with nodules the size of a pea. In addition, behind the peritoneum and in the mesocolon there were two tumors larger than pigeon's eggs. Neither sarcoma nor carcinoma could be demonstrated anywhere. The nodules in the peritoneum consisted exclusively of embryonic connective-tissue cells. Ewald draws the conclusion that teratoma of the ovary is in itself a malignant tumor, and that metastases can occur without carcinomatous or sarcomatous degeneration and without alteration of the histological structure.

I have not included in the foregoing list cases without recurrence or metastasis where it is stated simply that part of the stroma of the teratoma presented a sarcomatous character or nests of epithelium.

There are still some instances where teratoid tumors have undergone malignant degeneration, but they differ so markedly from the foregoing that they require separate mention. A few cases have been observed where flat-celled epithelioma has sprung from the skin lining the internal surface of what have always until lately been looked upon as simple dermoid cysts of the ovary. It would seem, however, that these presumably simple dermoid cysts are in reality complex tumors, for Wilms found in every case examined by him tissues representing all three embryonic layers. If all the ovarian dermoid cysts taken at random and examined by Wilms were compound tumors, one can justly assume with him that this is always their nature. But if all ovarian dermoid cysts are compound tumors, then all instances of malignant degeneration in such cysts would be

* *Wien. klin. Wochenschr.*, 1897, No. 10, p. 226. Abstract in *Centralbl. f. allg. Path. u. path. Anat.* ix (1898), 177.

instances of malignant degeneration in compound tumors. Although Wilms has found these dermoid cysts to be uniformly so complex, yet he does not believe that these ordinary dermoid cysts of the ovary should be classified with teratomata occurring in other parts of the body, and would consider them entirely apart and by themselves.

Epitheliomatous malignant degeneration has been reported as occurring in ovarian dermoid cysts by Heschl,* Biermann,† von Wahl,‡ Pilliet,§ Tauffer,|| Himmelfarb,¶ Krukenberg,** Veit,†† Wilms,‡‡ Klein,§§ Yamagiva |||| (2 cases), and Thumim,¶¶ thirteen cases in all.

In speaking of carcinomatous degeneration of ovarian dermoids, Thumim says that in looking over the literature he considers that the cases of Heschl, v. Wahl, Biermann, Himmelfarb, Krukenberg, Tauffer, and the second case reported by Yamagiva are the only undoubted examples of this affection which he has been able to find. Nine other cases that have been reported he cannot accept, partly on account of uncertainty that the disease was primary in the ovary, and partly on account of insufficient microscopical investigation.

In considering whether the adeno-carcinoma-like structures described in the case here reported were really cancerous or not, one must bear in mind that what appears to be identity of structure does not necessarily indicate identity of nature, and that structurally a resemblance to cancer may be simulated when one does not really exist. It does seem, however, that the adenomatous tissue here was malignant and therefore constituted an adeno-carcinoma. The epithelium was thrown about in a most disorderly fashion in alveoli having no basement membrane. Furthermore, these structures were heterolo-

* *Prager Vierteljahrsschr.*, lxviii (1860).
† *Prager med. Wochenschr.*, 1885, No. 21.
‡ *Petersburger med. Wochenschr.*, 1883, p. 70.
§ Cited by Wilms, *Deutsch. Arch. f. klin. Med.*, lv, 373.
|| Virchow's *Archiv*, cxlii, 389.
¶ *Centralbl. f. Gynäk.*, 1886, 569.
** *Arch. f. Gynäk.*, xxx, 245.
†† *Centralbl. f. Gynäk.*, 1890, 283.
‡‡ Loc. cit.
§§ Inaug.-Diss., Freiburg, 1893.
|||| Virchow's *Archiv*, cxlvii, 99.
¶¶ *Arch. f. Gynäk.*, liii, 547.

gous, for, normally, glands of this or any other character do not occur scattered at random throughout a living being, and heterology is an important element in the estimation of malignancy. The whole tumor was structurally embryonic in character, and looked as if rapidly growing, and clinically it was a rapid grower and recurrent; and embryonic structure, rapid growth and recurrence are of more weight even than heterology in arriving at a diagnosis of malignancy, but here all these combined in pointing to a malignant nature. Nevertheless it can be objected that metastasis was not proven, that infiltration of neighboring tissue was not a marked feature, and that the embryonic character and rapid growth of this glandular epithelium might simply have been co-equal with the embryonic character and rapid growth of the rest of this parasitic individual.

If, however, it be granted that the adeno-carcinoma-like structures in this case were really adeno-carcinomatous, then it is a very rare event indeed, so rare that Tauffer says no case of adeno-carcinomatous degeneration in a dermoid has ever yet been described.* Some of the structures in Jores' case, mentioned above, seem, however, to have struck the author as resembling adeno-carcinoma, for he describes some of the cysts as being irregular in shape and lined with cylindrical epithelium which did not rest on a basement membrane, but stood directly on the spindle-celled tissue of the stroma of the tumor. Metastatic nodules in the right lung were said to be exactly like the main tumor, and therefore, presumably, had also the above mentioned irregular cysts lined with columnar epithelium. In Emanuel's and in Lazarus' cases, already quoted, there were cysts in the metastases lined in the former with stratified epithelium and in the latter with goblet cells and cylindrical epithelium.

Some might assert their disbelief in the tissue removed at the second operation being a recurrent growth, principally because of the immense variety of structures found in it. They might say, that besides the three obvious lobes removed at the first operation there were probably other original implantation tumors scattered over the peritoneal surface that had not yet attained such a size as to attract attention, and

* Op. cit., p. 391.

that the tissue removed at the second operation was the tissue of original implantation tumors that had grown into prominence in the intervening time.

Against this opinion is to be urged what Dr. McNutt told me: that at the first operation the peritoneal cavity was, as far as he could see, perfectly healthy and free from tumor growth, excepting the area from which the original tumor grew; also that the large mass of the tissue removed at the second operation was taken from the site of the original tumor; that at the post-mortem the spread of the tumor had taken place as a continuous layer over the peritoneum; and that there were no indications at any point that this continuous layer of new tissue had been formed by the coalescence of smaller tumors.

The term "recurrent tumor" has been continually made use of throughout this article to designate the tissue removed at the second operation. By "recurrent," however, is meant that some particles of the original tumor, too small to be observed by the operator, were left behind and had grown rapidly so as to form the large tumor met with at the second operation. The probability that some of the original tumor was left unremoved at the first operation was shown by the discovery of the small piece of bone in the tissue extirpated at the second operation, for the time between these two operations seemed too short for fully developed bone to form.

A very small portion of the original tumor left behind and growing again would be sufficient to account for the great variety of tissues found in the recurrent tumor. To illustrate this more fully it is only necessary to relate that, at the suggestion of Dr. W. G. Hay, transverse sections were made of a bundle of the strings connecting the lobes of the original tumor. The diameter of none of the strings was greater than that of ordinary twine, but it was surprising to see the variety of structures, both epithelial and connective, encountered in each of them.

The incomplete removal of diseased tissue and the rapid reappearance of a tumor at the point of operation is, it is needless to say, a frequent and often unavoidable occurrence, and constitutes an important factor in the group of events to which the term malignant is

given. Malignancy in the present instance consisted of rapid growth, recurrence and the property of spreading by continuity. The tumor did not appear to have given rise to metastases, although from its structure this kind of malignancy might have been expected, and has been observed several times in similar teratomata.

Some of the different theories that have been framed in the past to account for the origin of teratomata may now be considered, and an opinion arrived at as to which one of them best fits the conditions found in the present instance.

I.—Many dermoids and teratomata are accounted for by a dipping down or involution of the external skin and subsequent pinching off. In such cases a cyst is formed with the epithelium of the involuted skin lining its inner surface. This theory does not, however, explain the present case, which was a solid teratoma covered by skin.

II.—Many teratomata admit of the explanation that they arise from a simple misplacement of a group of cells during development. The free situation in the abdominal cavity, the integumentary covering, and the great variety of structures in the case in hand would, however, all be against this view of its origin.

III.—Some authors have thought that irritation of a tissue may cause a compound tumor consisting of many varieties of the tissue irritated. This theory may be dismissed without comment.

IV.—What is called partial double formation sometimes takes place, that is to say, the same generative act that is responsible for the main being or individual also gives rise to a double formation of some of its parts. This theory satisfactorily explains the formation of supernumerary limbs, but hardly accounts for a tumor in which such diverse organs as skin, gut, nervous system and eye were found.

V.—At times evidently a complete double or twin formation takes place, with partial growing together or fusion of the twins. Many of the double monsters would appear to originate in this way, but the case in question is not to be so explained, for although there were two beings, no fusion had taken place between them. One individual simply grew inside the other.

VI.—It has been presumed that the unfecundated ovum can proceed

to develop without the assistance of the spermatozoon, and form an imperfect individual. The spermatozoon also without the concurrence of the egg is thought by some to have some formative power. This so-called parthenogenesis is supported by numerous examples in the lower orders of life, where the fecundation of the female is only required every few generations.

This possibility requires to be carefully weighed in the present case, as the egg is the only cell that we know of that can give rise to an individual having all three embryonal layers. And the converse is also true, that an individual having all three embryonal layers must arise from an egg. Therefore the individual under consideration necessarily originated from an egg. If this hypothetical egg came from the ovary of the child in whom the tumor was found, then this would be a case of parthenogenesis. This child, however, had not menstruated, and therefore presumably had not ovulated. It must be admitted, however, that it is thought that an egg may go on to development in the ovary before it has ovulated. But if this had been the case in the present instance the dermoid would have been found in the ovary, or would at least have been attached to it by a pedicle. As before stated the ovary was normal, and there were no traces at all of a pedicle or of a union between the tumor and the ovary.

VII.—It is held that two individuals may begin to develop from their respective ova, but before development is far advanced one may be enclosed by the other, and the included one's growth be so interfered with that it does not continue to develop but lies dormant for an indefinite time. This is called *fœtus in fœtu*, and is the most natural explanation of the facts in the present instance. It is easy of comprehension to suppose that during this little patient's fœtal life, and before the abdominal cavity closed, another egg, and possibly a fecundated one, slipped into the open peritoneal sac. The egg lay there as in a pocket up to the child's twelfth year, when it began to grow, but in a disorderly fashion and with all the energy of fœtal life. In this view the tumor would represent the patient's twin brother or sister.

NOTE ON DR. D. W. MONTGOMERY'S CASE OF TERATOMA.

By Lewellys F. Barker.

Through the courtesy of Dr. Montgomery I have been permitted to study sections from his tumor and to make (1) a note upon the nervous tissue present, and (2) some remarks concerning the theories which have been advanced to explain teratomata in general.

I.

The tumor contains large masses of nervous tissue distributed throughout its substance. While most of this nerve tissue resembles embryonic brain, including the lining of the central cavity with chorioid plexus, some medullated nerve fibres were discovered and also traces of embryonic eye structures. The masses of brain substance are scattered very irregularly through the tumor and at first sight apparently lawlessly. The size of the individual masses varies much, from small islands of the tissue recognizable only with the aid of the microscope to relatively large areas easily visible to the naked eye. Some of the masses are bounded definitely on one side by a cavity lined by cubical epithelium (rudiment of ventricles and ependymal epithelium), and on another side by vascular connective tissue (brain-membranes?). Other portions of brain substance are found for which no ventricular nor meningeal relations can be made out. In places the lining of the cavities supposed to represent the ventricles is seen to be made up of low, clear, cubical epithelium, which can be followed as a definite *lamina chorioidea epithelialis* over the much branched and anastomosing papillary strands of vascular connective tissue which project into the cavity, forming a distinct *plexus chorioideus* (Plate XXII, Fig. 13). No cilia could be made out, however, on any of the cells lining the cavities. In certain regions, instead of a lining with the characters just described, the tissue adjacent to the cavity resembles more closely that seen in certain regions in the embryo, for example that of the developing corpus striatum and of the developing spinal cord.

The ependymal epithelium is divisible into three distinct zones—an inner, middle, and external zone. In the middle zone of each cell is

placed the oval nucleus, which stains in hæmatoxylin, the nuclei of adjacent cells being placed at different levels, so as at first sight to give the impression of a mass of cells several layers deep. The internal and external zones of these cells are protoplasmic and stain intensely with eosin. The inner zone is narrow, and the outer zone, corresponding to the *Randschleier* of His and to the embryonic white matter, is much wider.

No cells which resembled adult nerve cells could be made out. Among the nuclei in the nerve tissue there were many small round rather solid nuclei resembling those of neuroglia cells; other nuclei were larger, only slightly oval and more vesicular. Some of the latter may be nuclei of neuroblasts, but the preservation of the tissue has not been such as to permit of a positive statement with regard to this point. Besides, nowhere in the masses of brain substance was it possible to make out definitely any axis cylinder processes of nerve cells. By far the most of what looks like brain tissue consists of a pale reticulum-like mass of fibrils (glia fibrils and processes of ependymal cells?), in the meshes of which are many small round cells with compact chromatin and scanty protoplasm (glia cells?).

Here and there embedded in the mass of pale glia-like tissue is to be seen a compact nodule, consisting of closely crowded small cells with scanty protoplasm and solid round nuclei. These nodules are not separated by any membrane from the surrounding tissue (Plate XXII, Fig. 12). What they represent it is difficult to say. One could think of masses of embryonic basal ganglia or simply of enormous aggregations of glia cells.

No traces of spinal ganglia such as Kappeler has described in one of his cases could be found. The only medullated nerve fibres observed were in the sections of skin. In these, several bundles of typical adult fibres with myelin sheath and neurilemma were made out.

As regards the presence of sense organs the tumor is notable in that it contains distinct evidence of rudimentary eye structures. Microscopic examination of the pigmented sheet of tissue found in the gross study of the tumor attached by one edge to a bone showed it to be derived from an incomplete eye. Dr. Montgomery states that one

surface was covered by a layer of flat, nucleated polygonal cells. The protoplasm of these cells is closely studded with fine brown, rod-shaped, pigmented granules (Plate XXII, Fig. 11). The cells are arranged in the form of a mosaic, and correspond to the stratum pigmenti retinæ derived from the portion of the ophthalmic vesicle not invaginated in the formation of the optic cup. No other part of the body resembles this structure. The layer of pigmented cells rests upon a very firm connective tissue membrane, which contains a few slightly pigmented connective tissue cells. No definite tunica vasculosa (chorioidea) could be made out, and the tough fibrous tunic adjacent to it, in places distinctly lamellated, probably corresponds to the sclera, though it would be difficult to prove this positively. In the original tumor no trace of the nervous portion of the retina nor of the optic nerve was discovered. In addition to the stratum pigmenti described, similar polygonal pigmented cells are to be seen irregularly distributed through the sections, sometimes apparently in solid portions of the tissues, sometimes lining small irregular slit-like spaces, and in one instance lining a space which is continuous with the cavity lined by ependymal epithelium, which probably corresponds to brain-ventricle. The latter observation is all the more interesting in that, as is well known, during development the cavity of the ophthalmic vesicle is continuous with the cavity of the central nervous system; the vesicle on each side arises as an outgrowth from the first cerebral vesicle at a point corresponding to the junction of the diencephalon with the telencephalon, marked even in the adult by a distinct pocket, the so-called recessus opticus. The pigment in all the cells mentioned was absolutely typical and unmistakable. There was no possibility of confounding it with hæmatoidin or with other pigments of hæmic origin.

The nerve tissues could not be studied by Golgi's method, as they had been hardened in alcohol. An attempt to impregnate pieces with bichromate subsequently proved unsuccessful.

If ear, nose and tongue were represented in the tumor, they either did not happen to be struck in the sections made, or, as is not unlikely, the tissues corresponding to them were too atypical to be recognized.

Masses of nerve tissue of the same general type as described above made up a large part of the recurrent tumor. Numerous areas were found in this in which there were traces of stratum pigmenti retinæ. The lining of some cysts consisted partially of such a pigmented layer.

The existence of peripheral nerves along with tissues of the central system appears to be rare in teratomata, though instances have been described by Baumgarten, Kappeler and others. It is interesting to consider the origin of the peripheral nerve fibres. In the cases in which well developed ganglion cells are present in the tumor one could think of the axones of the peripheral nerve fibres as direct processes of these cells, especially in cases such as Kappeler's, where well formed spinal ganglion cells were made out and photographed. In Dr. Montgomery's case no ganglion cells, at least none approaching in appearance the adult forms, could be found within the tumor. One could explain the origin of the medullated fibres in either of two ways: (1) by assuming the existence of ganglion cells in the new growth which were by accident missed in the microscopic study, or (2) by assuming that the nerve fibres represent ingrowths from the body of the patient herself, when the axones would represent prolongations of some of the nerve cells of the host. The latter view, if it be the correct one, need not necessarily be surprising, considering the recent findings of Young * concerning the presence of nerves in sarcomata and other tumors.

In view of the fact that masses of central nervous system, spinal ganglia and peripheral nerve fibres may be present in teratomata, it need not excite wonder that the organs of special sense are sometimes represented. Indeed it would be surprising were they absent.

II.

Many theories regarding the origin of teratomata have been brought forward. Among the best general discussions of the subjects are those of Meckel † and of Klebs.‡ The theory which suggests that such a tumor as the one described in the present article can result from the irritation of tissue cells in the region in which it arises may be dis-

* *The Journal of Experimental Medicine*, ii (1897), 1.

† Meckel, J. F., Handbuch der pathologischen Anatomie, 1812-1818.

‡ Klebs, E., Die allgemeine Pathologie, Theil ii, Jena, 1889, p. 797.

missed without further consideration. An ovarian or abdominal pregnancy is also out of the question, for besides the fact that no placenta existed, the child was *virgo intacta* and had not even menstruated. Moreover, teratomata may appear at any time of life, and in males as well as in females.

The doctrine of parthenogenesis and its various modifications, while supported by many able investigators in the past, is at present held by scarcely any one and need not therefore be discussed.

The theories which make the origin of teratomata congenital have most in their favor. But that in this case we have to deal with a tumor which has arisen from a dipping down or involution of the external skin with subsequent pinching off, as has been assumed for many teratomata, is clearly impossible. The variety of tissues present is too great, and the fact that we had to do, not with a cyst lined by skin on its inner surface, but with a solid teratoma covered by skin on the outside, excludes any such simple explanation.

One of the oldest of the modern theories regarding the origin of teratomata is that of Meckel, the theory of *foetus in foetu*. This theory, which makes the teratoma and its host twin individuals, accords best with the facts in the majority of instances and especially with those of the present case.

Klebs has divided the teratomata into (*a*) the *endogenous* forms, which represent tumors which have resulted from the pinching off of tissues during development, and (*b*) the *ectogenous* forms, which he believes to be always the result of fœtal implantation. As he says, however, the limits between the two must remain doubtful, and it is not impossible that many teratomata which have been included among the endogenous forms are in reality ectogenous teratomata which have undergone marked involution. The case before us would naturally belong to the ectogenous group of Klebs. His idea that in such cases we have always to do with two more or less separate fœtuses, one of which grows around and partly or wholly includes the other, seems a very probable one. He distinguishes two forms of ectogenous teratomata: (*a*) the allantoid variety, in which one fœtus is situated in one of the body cavities of the other, and its blood-vessels enter into communication with those of the including fœtus, and (*b*) the pre-

allantoid variety, in which there is a direct fusion, complete or partial, of the disc-like germinal areas of the two fœtuses at a very early stage, one finally gaining the ascendancy and growing around the other so as to include it. In the allantoid forms there is always an umbilical cord and sometimes a placenta; while in the pre-allantoid forms the umbilical cord and placenta are absent, and the included fœtus often shows very marked alterations and irregular development, probably corresponding to disturbances of vascular supply. In such instances the development may be so irregular and atypical that only microscopic examination reveals the true nature of the growth.

If we accept Klebs' hypothesis, then the case before us would agree best with the last mentioned group, and would therefore have to be considered as an instance of the abdominal form of the pre-allantoid ectogenous teratoma.

It is interesting to study the many transitional forms which are to be found between completely separated twin-formations and these complex teratomata * which may represent twins, one of the twins having included its fellow inside its own body. One has only to consult the atlases of Ahlfeld, Förster, Hirt and Piersol, Taruffi and others, to convince himself of large numbers and varieties of transitional forms. Thus instances in which one fœtus is partly inside and partly outside the body of a second are not at all uncommon. The well known examples of parasitic thoracopagi, of engastrius, epignathi, encranius, notomelus, pygomelus, etc., will be immediately recalled. Then there are forms in which one fœtus is entirely enclosed within the body of the second. It may be beneath the skin (inclusio subcutanea), it may be in the mediastinum, the abdominal cavity (inclusio fœtalis abdominalis), the sex glands, or even inside the skull. They are nearly always in situations in which embryonic inclusion can be easily accounted for. Some of these included forms are easily recognizable by the naked eye as independent individuals, but many of

* Marchand, in a masterly review of the whole subject of malformations, published in Eulenburg's Real-Encyclopädie der gesammten Heilkunde (3rd edition), although he places dermoids and teratomata in a group by themselves, states that he can make no fundamental distinction between them and genuine fœtal inclusions. He assumes, therefore, that both have a similar origin.

them are remarkably transformed and difficult to identify. They are frequently acardiac monsters, the mode in which the blood vascular relations in the one fœtus have become dominant at the expense of the heart and blood-vessels of the second having been well shown by Thoma in his Text-Book of General Pathology.*

It is usually assumed that in fœtal inclusions we have to deal with two eggs, separately fertilized. As has been suggested by Sobotta, Marchand and others, it is not impossible that one or more "directive corpuscles" become fertilized and give rise to an embryo, since it has been proven by Mark, Bütschli and Boveri that they represent abortive eggs, and Platner has actually seen such structures penetrated by spermatozoa.

That the included embryo should remain dormant (in the present case for twelve years, in other instances in the bibliography much longer), and after the lapse of a long time give rise to serious symptoms, is difficult to explain satisfactorily, but it must be remembered that as yet our knowledge of the conditions underlying the mechanics of development is extremely meagre. We have evidence in comparative embryology which permits the statement that a fertilized egg may be placed under conditions which preclude cell-division, and that it may later under more favorable circumstances go on to normal development.

The question as to the possible origin of such a tumor as a consequence of splitting in the long axis of a single embryo at a very early stage must not be overlooked. In view of the results of modern experimental teratology such an origin cannot be excluded. Certainly many of the cases of anadidymi, katadidymi and the like are best explained by assuming a partial splitting at an early stage. The objection that formation of double parts or extra organs, or even an entire second embryo, would necessarily lead to gross defects in the development of the original embryo, no longer holds, since Driesch, Wilson and Morgan have demonstrated the remarkable regenerative power of the very young embryo after injury (Roux's post-generation). We know now that in many animals, after fertilization and segmentation, a whole embryo may develop from each of two segmentation-cells iso-

* English translation by Alexander Bruce, London, 1896, vol. i, p. 183.

lated by shaking. Even in the eight-cell or sixteen-cell stage, small but complete larvæ may develop from a single cell if it be isolated from its fellows. Of more than ordinary interest is the observation of Roux who noticed such isolated segmentation cells inside the bodies of young frog larvæ and suggested that they might be of significance in the etiology of tumors. The curious admixture of tissues, the marked excess of certain elements and the deficient development of other parts in teratomata need not surprise us when we consider the extraordinary disturbances to which the developing tissues in many of these cases are exposed.

No matter what view we take concerning the mechanisms of development and tissue-differentiation, be it Roux's modification of the evolutionary conception of Weissmann, or the more epigenetic doctrine of Hertwig, or a compromise between the two as suggested by Benda, there can be but little doubt that in the most complicated teratoma, where the arrangement of tissues is apparently most lawless, there is in reality no condition present which dare be considered as accidental. The relations which exist are the result of the response of activities inherent in the included embryo (and possibly activities which in the beginning were entirely normal) to the extraordinary influences from the environment to which such an embryo is subjected, and of the consequent manifold alterations of correlations inside it.

Concerning the nature of the primary causes which can lead to such a splitting in one embryo, or to the establishment of relations between twin-embryos which are destined to be disastrous to one and sometimes (perhaps only after years have passed) to both of them, we are as yet almost completely ignorant. The experiments of Newport, Panum, Valentine, Dareste, Gerlach, Pflüger, Roux, Born, Loeb, Hertwig, Ballantyne, Morgan, E. B. Wilson and others have in the field of comparative teratology demonstrated the effects of alterations of temperature and position, or of chemical relations such as modification of the oxygen supply, or the introduction of toxic substances, the influence of polyspermia, of pressure, shaking, and operative insult upon fertilized eggs and upon embryos in very young stages. While we dare not transfer directly the results of these experiments to the domain of human teratology, still the mass of data

which they have afforded concerning the possibilities of developing cells may, along with further experiments in the same field and careful studies of pathological human embryos, open up the way which will lead ultimately to the construction of a satisfactory hypothesis concerning the nature and mode of origin of malformations and teratomata in human beings.

That teratomata, though undoubtedly of early embryonic origin, may appear at almost any age in life; that many show inside them tissues far removed from the normal type, sometimes one form of tissue predominating, sometimes another; further, that the mode of their growth and termination varies exceedingly; and finally, that metastases undoubtedly occur in certain instances—all these facts taken together lend support, it must be granted, in a very striking way to the doctrine of Cohnheim regarding the origin of tumors in general.

DESCRIPTION OF PLATES XIX–XXII.

PLATE XIX.

Fig. 1. Photograph of the original tumor.

Fig. 2. Skin of the original tumor.

PLATE XX.

Fig. 3. Part of cyst-wall showing goblet cells. Original tumor.

Fig. 4. A tube lined with cuboidal epithelium and surrounded by spindle-celled tissue in which islands of cartilage lie scattered. From the original tumor.

Fig. 5. Showing hair follicle from the original tumor. The first layer of cells lying on the basement membrane of the hair follicle have their nuclei far removed from the base of the cells.

Fig. 6. Bed hair from the original tumor.

Fig. 7. A typical epithelial tissue resembling carcinomatous infiltration.

PLATE XXI.

Fig. 8. Embryonic intestine provided with developing villi and surrounded by smooth muscle. Original tumor.

Fig. 9. Metaplasia of columnar epithelium into flat-celled epithelium with pearl formation. Recurrent growth.

Fig. 10. Pearl formation in recurrent growth.

PLATE XXII.

Fig. 11. Pigmented polygonal epithelium resembling that of the retina. Original tumor.

Fig. 12. Section showing embryonic nervous tissues. Recurrent growth.

Fig. 13. Section showing embryonic nervous tissue (glia) (C. n. s.) and cavity lined with ependymal epithelium and choroid plexus (Pl. ch.).

The drawings, with the exception of Figs. 8 and 13, were made by I. Katsuki, M. D., Univ. of California.

FIG. 1.

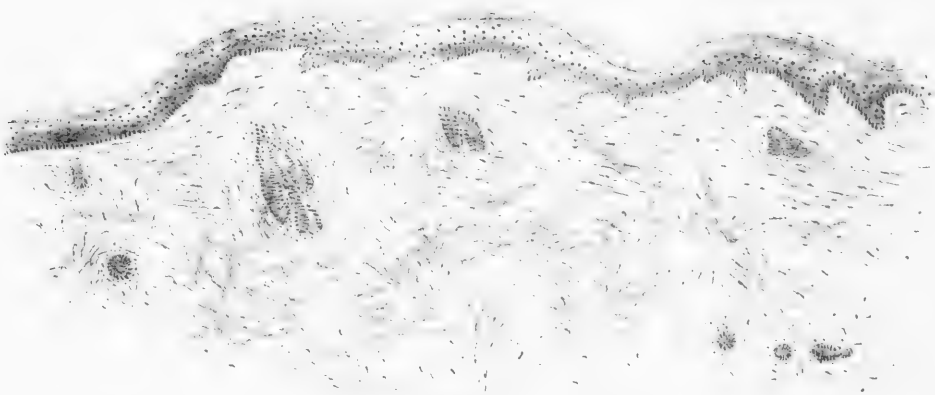


FIG. 2.

FIG. 3.

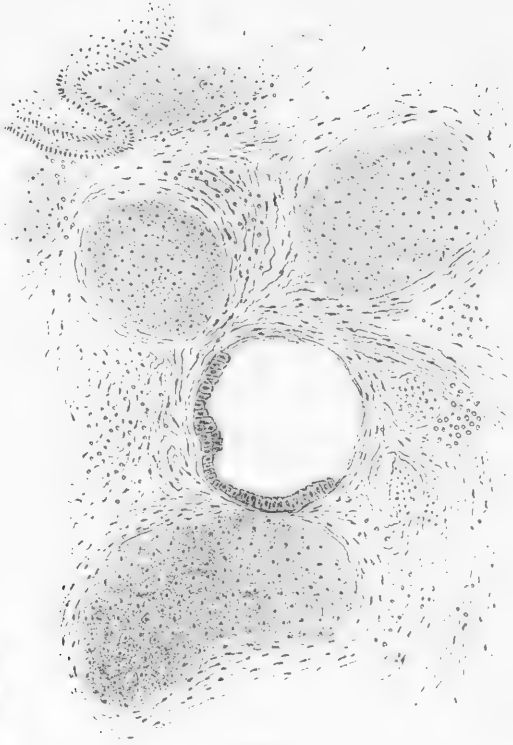


FIG. 4

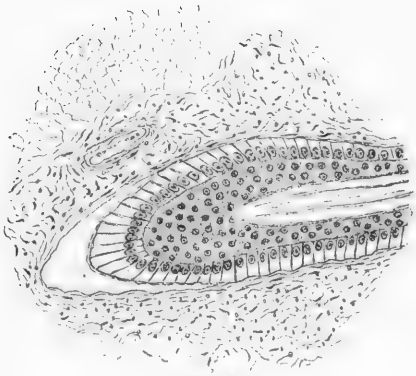


FIG. 5.

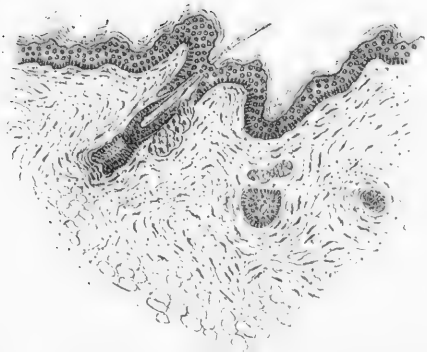


FIG. 6.

FIG. 7.

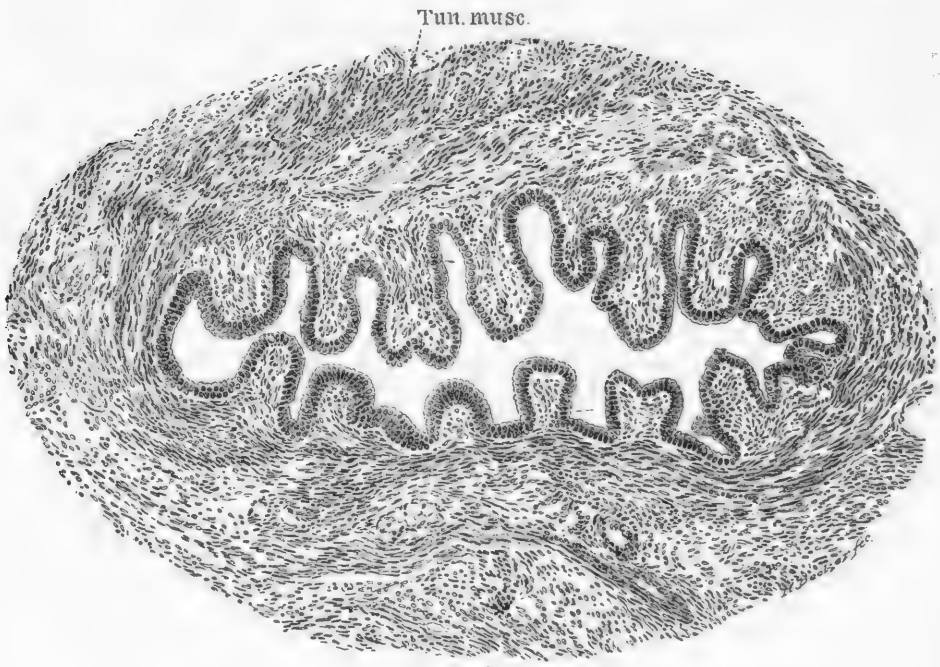


FIG. 8.

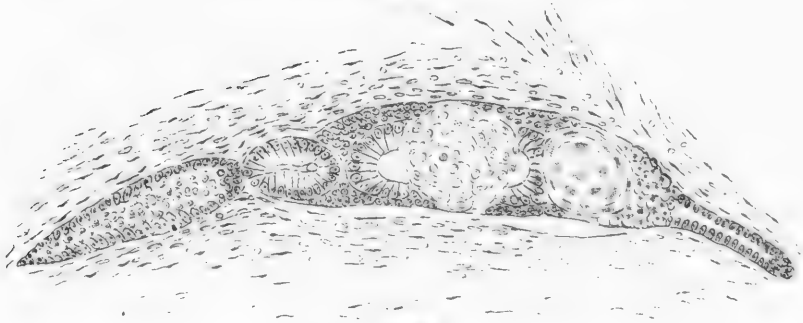


FIG. 9.

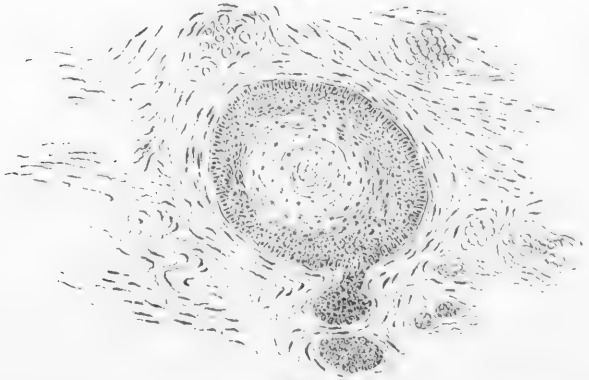


FIG. 10.

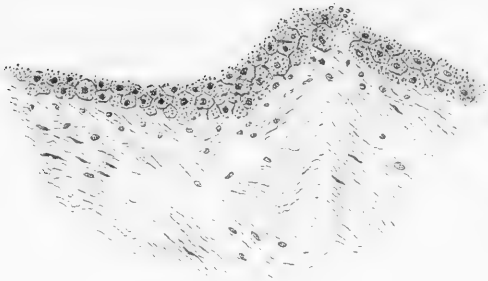


FIG. 11.

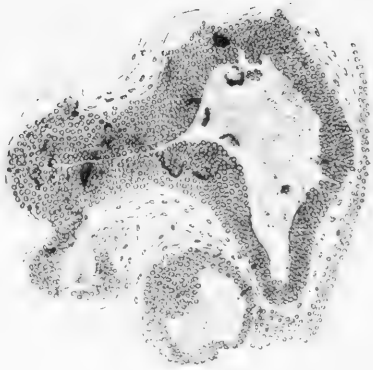


FIG. 12.

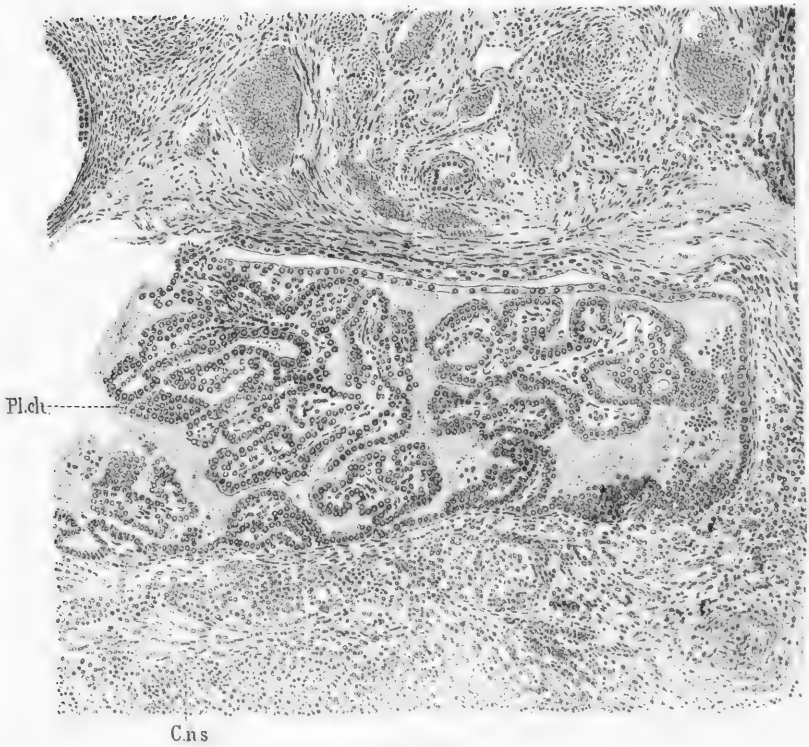


FIG. 13.

AN EXPERIMENTAL STUDY OF FAT STARVATION WITH ESPECIAL REFERENCE TO THE PRODUCTION OF SEROUS ATROPHY OF FAT.

By C. A. HERTER, M. D.

Plates XXIII and XXIV.

The experimental study which forms the basis of this paper was originally undertaken to determine whether the lesions of rickets or similar lesions can be produced in growing animals by withholding fats from their food as far as practicable. This inquiry was suggested by the alleged frequently low fat content of the milk of women whose children have grown rachitic on breast-milk, by the occurrence of rickets in children fed largely on condensed milk, which is poor in fat, and by the fact that the clinical indications of rickets are often lessened by a diet rich in fats.

The common pig, Sus scrofa, was the animal chosen for the experiment, partly because of his omnivorous habits and partly because the nutritive vigor of this animal seemed likely to render possible the extension of the necessarily depressing experiment over a long period of time.

CLINICAL SECTION.

Pig 1.—The first experiment was begun December 16, 1895, upon a boar about two months old weighing 24 pounds, and which will be referred to here as pig 1. This animal was fed exclusively upon milk furnished by the Walker-Gordon Milk Laboratory, from which the fat had been largely removed by the centrifuge. The composition of this milk as regards fat is remarkably constant, and analysis shows that it contains less than $\frac{1}{2}\frac{1}{0}$ per cent of fat. The milk of the sow normally contains a high percentage of fat, the amount ranging from 8 to 10 per cent. The usual content of cow's milk skimmed by the ordinary method is about 1 per cent. The milk fed to pig 1, therefore, contained less than $\frac{1}{2}\frac{1}{0}\frac{1}{0}$th part of the fat which is found in sow's milk, and about $\frac{1}{2}\frac{1}{0}$th part of the fat contained in ordinary skimmed milk. In other respects the milk did not differ greatly in composition from

that of cow's milk, the content of milk-sugar being about 6 per cent, that of proteids about 4 per cent.

The duration of the experiment was 51 weeks. The daily quantity of milk allowed first was 1400 cc. This was increased from time to time until at the end of 46 weeks the total daily amount was 4000 cc. The feeding was usually in two portions, towards the end often in one. During the first six weeks the initial weight of the animal was just maintained; then during a period of about six weeks there was a moderate loss, the weight dropping from 24 to 19½ pounds. After this an increase in the food supply was followed by a steady gain in weight, so that 12 weeks after the beginning of the diet the animal weighed 2½ pounds more than at the beginning. 16 weeks from the beginning the weight showed an increase of 5 pounds, in 20 weeks an increase of 7½ pounds, and in 50 weeks an increase of 16 pounds, in all from 24 to 40 pounds. Within a week from the beginning the fæces were observed to be lighter in color and firmer in consistence than when the animal was on a mixed diet containing cereals. Very soon there was distinct constipation and the fæces were passed as small hard spheroidal masses. This constipation continued to the last. The fæces were regularly alkaline in reaction. The quantity of urine passed varied closely with the amount of milk given. The specific gravity varied between 1011 and 1014 in 24-hours' collections. The reaction was invariably distinctly acid—a notable peculiarity, as the urine of pigs on a general diet is almost always neutral when passed. During the first 8 or 10 weeks the animal seemed to be in every way normal except that he did not gain in weight. After 18 weeks it was observed that the animal's hair and skin were distinctly drier than is usual in pigs of this age, and that the hair was decidedly more scanty than that of a control pig of the same age. After 30 weeks these conditions were more pronounced, and some weakness of the hind legs was noted when the animal stood. From about this time it was noticeable that the animal lay on the floor of his cage a good deal of the time, and often dozed during the day, though he did not usually sleep. After 50 weeks he was quite unable to stand on his hind legs, which were thrown forward as the animal lay. There was no attempt whatever to use these extremities in walking. Some power remained in the hip muscles. The muscles were flabby but showed no indication of wasting. On the contrary the legs were puffy in appearance, although there was absolutely no pitting on pressure. This condition had doubtless been present some time before it was noted. The knee-jerks were present. The forelegs were weak, but the animal was able to walk by use of them. For a few days before his death the

animal had a rise in temperature, his breathing was rapid and labored and he was cyanosed. When it was evident that death was impending he was killed. An examination of the blood just before death showed the hæmoglobin to be reduced to 65 per cent by Fleischl's instrument. The proportion of small red blood cells seemed increased. The proportion of white to red cells was normal. The blood count showed a slight reduction in the total number of red cells to the cubic millimetre.

Pig 8.—A second experiment was conducted with another boar weighing 26 pounds, and referred to here as pig 8. This animal was fed exclusively upon milk of the same composition as that used in the case of pig 1, but was given during the first twelve months of the experiment as much of the food as he was able to take, instead of being limited to a fixed quantity. The quantities consumed by him were considerably in excess of the amounts allowed pig 1—5, 6 or 7 litres per day. Toward the end of the first year the quantity of milk consumed was from 8 to 10 litres daily; from the end of the first year until the termination of the experiment the quantity was reduced to two litres per day. The duration of the experiment was 66 weeks. At the end of 12 weeks the skin was observed to be dry and scaly, and the hairs were scanty. At the end of 20 weeks some weakness of the forelegs was shown in standing, and the animal was inclined to sleep a good deal of the time. After 30 weeks there was marked weakness in all four legs. The appetite remained good. The urine and fæces showed the same peculiarities as were noted in the urine and fæces of pig 1. From time to time in the course of this experiment bits of skin and subcutaneous fat were removed from the back of the neck in order that their structure might be compared with a piece removed before the animal was put on this diet, and with the skin of pig 1. The weights of the animal were as follows: Nov. 12, 1895, 28 pounds; Jan. 21, 1896, 42 pounds; February 29, 55 pounds; April 21, 62 pounds; Nov. 23, 106 pounds. The gain in weight was thus much greater than in pig 1. On the reduction of the milk to 2 litres daily there ensued considerable loss in weight, and one week before death the animal was quite unable to stand owing to weakness. He was killed by bleeding.

Pig 9.—A third experiment was conducted on a pig weighing 24 pounds, with a view especially to a study of the chemical characters of the urine and fæces during the starvation. After 6 weeks the animal was again allowed an abundance of fat, and after two weeks this was again cut off. This animal is referred to as pig 9.

Pig 2.—At the time when pig 1 was put on a fatless diet, another animal, here known as pig 2, of about the same age and weight, was

placed upon a wholly different diet, in which the quantity of proteid and of fat was much reduced, but in which an attempt was made to compensate for their reduction by the use of very large amounts of carbohydrate food, first in the form of Mellin's food, later as unrefined sugar. The results obtained from the study of this animal bear directly on the question of fat starvation, and in some ways the experiment may be regarded as a control to the others. The duration of this experiment was 4 weeks longer than in the case of pig 1, namely, 56 weeks. There are several respects in which a comparison between the two animals is instructive. The urine, instead of being distinctly acid, as was regularly the case with the urine of pig 1, was neutral or alkaline at first, and later slightly acid in reaction. Toward the end of the experiment the urine at times contained sugar in small amounts soon after the administration of the richly saccharine food. The fæces, instead of being hard in consistence and light in color, as in pig 1, were usually dark in color and semi-fluid in consistence, and part of the time were diarrhœal in character.

More important clinical differences relate to the general condition of the animals. Although there was an even greater retardation of growth in pig 2 than in pig 1, in the former the skin remained soft and apparently well nourished, though a little dry. There was some falling out of the hair, but that which remained was abundant in comparison with the hair of pig 1. The animal appeared fairly well nourished, though small. There were no hæmorrhagic spots in the skin. A few days before the animal was killed he seemed rather feeble in all four legs, as shown by difficulty in standing, but he developed no distinct paralysis. There was no swelling of the feet as in the case of pig 1.

To sum up: it was found that a growing pig which lived exclusively on nearly fatless milk for about one year was retarded in growth, suffered markedly in nutrition, and developed a hæmorrhagic eruption* and marked weakness of the extremities; that another pig kept on the same diet for 66 weeks developed the same clinical conditions excepting hæmorrhagic spots, but in less marked degree; and that a third animal which lived on a diet much restricted in proteids and fats but with a great excess of carbohydrates, though more retarded in growth, suffered distinctly less in general nutrition and apparently in a different manner in several respects.

* It seems not unlikely that certain of the lesions noted in this animal, especially the hæmorrhagic ones, were scorbutic in character.

PATHOLOGICAL ANATOMY.

Skin and Adipose Tissue of Pig 1.—The body is poorly nourished; there is a fairly abundant hæmorrhagic eruption on the lower part of the abdomen and into the skin of the perineum. Over the upper part of the sternum is an ulcer about 2 cm. square (clearly from pressure). On cutting through the skin it is seen that there is nowhere any subcutaneous fat; in place of this is a homogeneous, translucent layer of gelatinous-looking material, somewhat less thick than the layer of subcutaneous fat in a normal pig of the same size. On the back of the neck, near the median line, where the fat is normally very thick, this material forms a layer about 22 mm. in thickness. It may be stated here that in all parts of the body where fat is normally present a similar replacement of it by this material is evident, as about the heart, about the kidneys, between the muscles, in the omentum and elsewhere. At first sight the appearance of the material suggests that of œdematous connective tissue, but the substance appears quite homogeneous and yields no fluid on being cut into small pieces and compressed. This replacement of fat by the material described is the most striking and important pathological change noted in this animal. The microscopic characters of the skin are normal. The subcutaneous fat-cells are found to be so transformed as to be almost unrecognizable. The gelatinous layer consists chiefly of spheroidal and ovoidal cells (Plate XXIV), the average diameter of which is from one-third to one-sixth that of normal fat cells (Plate XXIII). The cells consist of a cell membrane, frequently irregular in outline, containing a homogeneous substance which stains rather faintly with hæmatoxylin and most basic aniline dyes. Each cell contains a nucleus which stains deeply with hæmatoxylin and resembles that of a young fat cell. These nuclei are not applied closely to the cell membrane, but may occupy any portion of the cell body. The intranuclear structure was not studied. Often the nucleus is surrounded by a small or considerable amount of finely granular material. Not infrequently what appears to be a vacuole of considerable size is found in the cell body. Sections from material subjected to the action of osmic acid, before further hardening and embedding, show no evidence of the presence of fat—they are wholly unaffected by this reagent. Running in from and continuous with the connective tissue of the skin are bundles of various sizes formed by connective tissue fibres. Frequently the smaller bundles separate into the individual wavy elastic fibres of which they consist, and these pass in all directions between the cells.* The

* The elastic fibres are very abundant, but this is normally the case in the skin from the region of the ligamentum nuchæ.

cells do not lie close together as in normal fat tissue, but are separated from one another in places. Their appearance suggests that the intercellular spaces may have been occupied by some material which has been lost during the process of hardening or embedding. Between the cells with unbroken cell membranes sometimes lie processes which may consist of broken cell membrane. The capillaries are fairly numerous. Some of them have blind ends. Unfortunately the material from pig 1 was not examined fresh.

Before describing other changes noted in pig 1 it will be convenient to refer to the changes found in the skin in pig 8 and in pig 2.

Subcutaneous Fat in Pig 8.—In this pig, skin from the back of the neck was removed from time to time. After nine weeks it is noticeable that there is some shrinkage of the fat cells, and that the cells in places are irregular in form. After fourteen weeks the fat cells are shrunken to two-thirds or one-half of their normal size; the outline of the cell membrane is often exceedingly irregular; the cell contents consist of larger and smaller droplets of fat which stain well with osmic acid, and it is noticeable that some of the nuclei are no longer in apposition with their cell walls. Twenty weeks after the beginning of the diet the fat layer appears pale. Although it does not look quite like normal fat, it does not appear gelatinous. The fat cells show more reduction in size than in the preparations referred to above. This is more pronounced in some lobules than in others. A few cells are seen which are not more than one-quarter or one-fifth the average diameter of normal cells. In preparations cut from pieces immersed in a 1 per cent solution of osmic acid the fat cells nearest the edge of the section have stained like normal fat cells, but there is a line of cells a little farther from the edge to which the osmic acid has penetrated less freely. Here the contents of the cells have stained only in spots, corresponding to the fat droplets already referred to. Between the droplets is seen in places a very finely granular or homogeneous material which does not stain with osmic acid, but tinges faintly with hæmatoxylin. This certainly is not fat, and it is possible that it is the accumulation of such material in larger amounts both in and between the cells which gives rise to the gelatinous appearance noted in pig 1. The nucleus in many of the cells lies well within the cell body. The connective tissue which lies between the groups of cells shows a distinct inclination to the separation of the individual fibres of which it consists—an unraveling, as it were, similar to but much less pronounced than that observed in pig 1.

The only additional change observed in the subcutaneous fat of this animal at the end of 60 weeks is a further considerable reduction in the

size of the fat cells. In some places the subcutaneous fat has almost wholly disappeared; in most places it is from $\frac{1}{8}$ to $\frac{1}{4}$ in. in thickness. Nowhere has there been any replacement of fat by distinctly serous material. A majority of the fat cells are simply much atrophied, but a few show the alterations described as present after the lapse of 20 weeks.

Subcutaneous Fat in Pig 2.—An examination in this case shows that the skin itself is normal in structure. The subcutaneous layer of fat is much reduced in thickness, being only about one-quarter the thickness of the fat layer from the corresponding portion of the back of the neck in a normal pig of the same age. The gelatinous appearance which was noted in the skin of pig 1 is wholly wanting, but the fat is perhaps a little paler in tint than that of normal animals. Minute examination shows that the fat cells are somewhat reduced in size, perhaps to two-thirds or three-quarters of their normal diameters. The contents of the cells are everywhere homogeneous; nowhere is there to be seen the breaking into fat globules noted in the sections from pig 8. The cell contents stain faintly with hæmatoxylin. With osmic acid they stain exactly like normal fat cells. The connective tissue separating groups of fat cells is quite normal in appearance. Between the individual fat cells are more nuclei than are normally present in an animal 15 months of age, but not more than are seen in the subcutaneous fat of pigs a few weeks old.

The appearances, then, are chiefly those which we might expect to find as the consequence of the arrest of fat formation in a young animal. The reduction in the size of the cells is doubtless to be regarded as the result of a process of simple atrophy. Notwithstanding the long period over which the experiment extended, there is nothing to suggest even the beginning of the transformation which was noted in pigs 1 and 8.

Examination of Pig 1 continued.—On opening pig 1 much straw-colored fluid is found in both pleural cavities, in the pericardium, and in the peritoneal cavity. The parietal pleura on both sides is the seat of a moderate number of small superficial hæmorrhages; the lower lobe of the right lung is partially consolidated, and on section presents numerous larger and smaller infarctions. On the pleural surface over the consolidated area is a thin layer of fibrin. The base of the left lower lobe is congested, but there is no consolidation. Microscopically the consolidated lung presents numerous infarctions with surrounding pneumonic consolidation. The heart is large, pale in color, and flabby in consistence. The weight of the heart is 232 grammes. That of a normal animal of the same age, and weighing

five and one-half times as much, is only 358 grammes, or about one-half as much more. On the anterior surface on either side of the coronary artery are several hæmorrhages of moderate size, extending about one-quarter of an inch into the myocardium. In place of the subpericardial fat usually found is a layer of the gelatinous tissue above described. On section the myocardium is pale and the wall of the left ventricle is notably thickened. The valves are normal. There are several small endocardial hæmorrhages in the left ventricle. The coronary arteries were not examined. Microscopic examination shows that many of the muscle fibres of the left ventricle are the seat of slight granular degeneration. In places there are small areas of richly cellular connective tissue. The liver is normal in appearance and consistence. The gall-bladder is distended with light-colored bile. The bile duct is pervious. The histological appearances of the liver are normal. The mucous membrane of the stomach is normal in appearance. In the cardiac portion there are a few hæmorrhagic spots in the mucosa, and one small superficial ulcer. The mucous membrane of the large and small intestine is normal in appearance. The pancreas is normal in consistence, yellowish in color and disproportionately large in comparison with the body weight. Histologically it appears to be normal. The spleen is small, rather pale, and normal in structure. The kidneys are surrounded by a considerable amount of hyaline gelatinous material of the same character as that beneath the skin and representing altered adipose tissue. The kidneys are disproportionately large in comparison with the body weight. The capsules strip off readily. The surfaces are smooth, slightly paler than normal; the striations are almost obliterated and the cut surface is studded with numerous minute hæmorrhages. The chief histological changes observed in the kidney are as follows: The epithelium of the tubules is everywhere the seat of granular degeneration, the changes being slight but distinct in the collecting tubes and in Henle's loops, and considerable in degree in the secreting tubes. The cells of the secreting tubes are somewhat swollen and in some cases desquamated. Many degenerated cells fill the tubes. In other cases the contents of the tubes consist of the nuclei of degenerated cells and a granular detritus. Frequently the secreting tubes contain hyaline material which stains with eosin. Nowhere do the tubes seem much dilated. The tufts are somewhat swollen and usually occupy the entire capsular space. They appear more richly cellular than normal. The epithelium covering the tufts is in places swollen and granular, that lining the capsule appears normal. The capillaries of the tuft generally appear compressed. The vessels everywhere seem

normal. No alterations can be detected in the connective tissue. In short, the appearances are those of a chronic parenchymatous degeneration. The adrenals are normal in consistence but are distinctly increased in size, their weight being as great as in a normal pig whose body weighs more than five times as much as that of pig 1. On section the cortex appears thickened and the medulla is abnormally dark, suggesting the presence of hæmorrhage, but this view is not confirmed by microscopic examination. The radially disposed columns of cells in the cortex appear swollen, and in places the cell bodies are less granular than normal. The nuclei seem unchanged. In the medullary portion the ganglion cells have become greatly swollen and in some cases are almost wholly replaced by large vacuoles. Through an oversight the thyroid was not removed.

The knee-joints are found to be filled with bloody and viscid synovial fluid. The bones of the skull are porous, very brittle and distinctly thinner than those of a normal animal of the same size. The femurs are about normal in weight. The bone marrow in each femur is replaced wholly by material which has the appearance and consistence of a blood clot. Unfortunately this material was not successfully hardened, and no satisfactory examination was made of it. The remaining long bones were not examined.

The central nervous system was not examined histologically. Of the skeletal muscles pieces only of the quadriceps femoris were examined histologically. Sections of the muscle show that a majority of the muscle fibres have undergone a marked reduction in size. While there is an apparent increase in the sarcolemma nuclei, there is probably no actual increase in their number. A few fibres are reduced to a width not greater than the diameter of their nuclei. The striations of the fibres are as a rule well preserved. The fibres stain well with eosin, and on transverse section some of them have a vitreous appearance and look as though broken into large fragments. There is no increase in connective tissue. The intermuscular fat shows the same peculiarities that have been noted in the case of the subcutaneous fat. All the visible vessels show an increase in the thickness of their walls, which is in some cases very marked and implicates chiefly the muscular coat.

The post-mortem notes on pig 2 are as follows: There is little or no subcutaneous fat; the lungs are normal; the heart is normal in size, color and consistence; the muscles are a little soft; the liver is normal in appearance and consistence; the pancreas is rather hard; the kidneys are normal; the striations of the cortex are very plain. The femur, cut longitudinally, shows the development of bone to be almost exactly the

same in degree as in the case of pig 1. In thickness and consistence the bone is not perceptibly different from that of pig 1. The marrow adjacent to the compact bone is red in color; it has the appearance presented by *all* the marrow of pig 1, whilst the remaining and greater part of the marrow, that occupying the central portion, is of the normal light pink color. The hæmoglobin was estimated immediately after death. The reading with Fleischl's instrument was from 100-105 per cent.

Examination of pig 8.—The animal is considerably emaciated. The subcutaneous fat is everywhere either entirely absent or greatly reduced in amount. The muscles are normal in color and consistence. The pericardium contains almost two ounces of clear straw-colored fluid. The heart is flabby in consistence and pale in color. Hæmorrhages into the heart muscle are not noted. The liver, spleen, pancreas and kidneys appear normal. The epi- and pericardial fat presents the same gelatinous appearance observed in pig 1. The same change is very pronounced in the adipose tissue about the kidneys and the ureters and in the omentum. Microscopic examination shows that the atrophic adipose tissue about the heart, kidneys and elsewhere presents essentially the same appearances as those already described as occasionally present in the adipose structures of pig 1. The only difference worthy of mention is that there is visible an abundant homogeneous intercellular matrix, which probably consists of an albuminous serous material. Aside from the alterations in the adipose tissue the following changes are noticeable. The skeletal muscles show granular degeneration of the muscle fibres in places; other fibres exhibit different grades of simple atrophy. In the cortex of the pancreas are numerous hæmorrhages of moderate size, and in places free brown granular pigment. In some areas the epithelium is the seat of granular degeneration. The secreting tubes of the kidney show marked granular degeneration of the epithelium; they contain some granular debris and fibrin, and some hyaline and granular casts. The epithelium of the loops and collecting tubes shows granular degeneration; the tubes contain much hyaline material and some granular casts. The capsular epithelium is swollen and slightly degenerated. Sections from the motor cortex stained with eosin and hæmatoxylin and by Nissl's method show degenerative changes in a considerable proportion of the nerve cells. The outer layers of the cortex are œdematous. The cortex of the frontal region shows similar changes. In several places there are small hæmorrhages into and beneath the pia of the central and frontal convolutions.

In the case of pig 1 an endeavor was made to determine the nature of the subcutaneous gelatinous material which has been described. With a view to determining the presence of mucin the tissue was extracted with lime water, mucin being soluble in dilute alkalies. It was found that on the addition of dilute acetic acid to this extract there occurred a considerable flocculent precipitate. Up to this point the behavior of the material is that of a mucin-containing substance. When, however, a 2 per cent solution of acetic acid was added to the precipitate the latter was again dissolved. In this respect the extracted material no longer corresponds to the reactions of mucin. Its solubility in a 2 per cent solution of acetic acid is a characteristic which removes it from the group of mucins, which are not soluble in an excess of acetic acid. Moreover, on decomposition with dilute sulphuric acid it failed to yield a reducing substance such as is obtained in the case of mucin. The mucins are glucosides, that is, compounds of a true proteid (generally a globulin) with animal gum, which by treatment with dilute sulphuric acid are converted by hydration into a reducing substance. On the other hand the lime water extract gave the characteristic xanthoproteic reaction and the reaction with Millon's reagent. This is conclusive evidence that the body under examination is not, strictly speaking, a mucin, but is a true proteid, and probably places it in the group of phosphorus-containing proteids known as nucleo-albumins. Unfortunately the phosphorus content of the material was not determined. Positive proof that it is a nucleo-albumin is therefore wanting.

Before discussing the subject of fat atrophy we may pass to the consideration of some nutritive disturbances which have been noted in the animals that were subjected to fat starvation. As already stated, there was some reason to think that fat starvation might lead to the production of rickets or a condition resembling rickets. For this reason attention was paid to the study of the salts of the urine and fæces, and the results noted were of sufficient interest to induce a continuation of this class of observations even after it became clear that the lesions produced were not those of rickets.

Looking first at the urine, we find that in the case of pig 1 on Dec. 24, 1893, one week after the beginning of the fatless diet, the urea excreted during the 24 hours was 8.047 gm., whilst the P_2O_5 excreted in the same period was 1.175 gm., the ratio of P_2O_5 to urea being 1 to

6.8. The ratio in health, on a milk diet, varies from about 1 to 6 to 1 to 14. On Jan. 4, 1894, the urea was 18.142 gm., the P_2O_5 1.190 gm., with a ratio of 1 to 15.2. On January 30 the urea was 30.895 gm., the P_2O_5 1.023 gm., ratio 1 to 32.4. On April 20 the urea was 25.039 gm., the P_2O_5 1.065 gm., ratio 1 to 23.5. Finally on Nov. 24, 1894, a few days before the completion of the experiment, the urea was 38.626 gm., the P_2O_5 0.759, ratio 1 to 50.9. Looking these figures over it will be seen that there was a very considerable increase in the amount of urea excreted between the beginning and end of the experiment, an even greater increase than was to be expected in a young and growing animal. But while this occurred in the urea excreted there was not a corresponding increase in the excretion of P_2O_5. Far from there being any increase in the absolute amount of P_2O_5 excreted, the figures indicate an inclination to an absolute decrease. Under ordinary conditions both in pigs and in man the excretion of P_2O_5 shows a rough correspondence with the urea excretion in any fluctuations that may occur. In the diet of pig 1 the P_2O_5 of the milk was increased proportionately with the proteids of the milk. Hence we should expect to find a more than ordinarily close correspondence between the excreted urea and the P_2O_5. On the contrary we have ratios fluctuating from 1 to 6.8 to 1 to 50.9.

The observations on pig 8 were unfortunately not begun until the fatless diet had been continued for nearly 8 weeks. There is lacking, therefore, a study of the early period in which it is likely that the most pronounced deviation from the normal ratio of P_2O_5 to urea might be expected to occur. The results are, however, not without interest. Between January 9 and April 8, the period of observation, the urea fluctuated irregularly between 20.38 gm. and 42.64 gm. daily, whilst the P_2O_5 varied from .71 gm. to 1.93 gm. The ratios fluctuated irregularly between 1 to 16.70 and 1 to 48.77, with no material difference between the ratios at the beginning and at the end of the period, but with perhaps an inclination to lower ratios at the end than at the beginning. The striking feature of the observations is the high ratios throughout, or in other words the small amount of P_2O_5 in proportion to the urea.

An examination of the ratios in the case of pig 9 leads one to suspect that in pig 8 there may have been a larger excretion of P_2O_5 at the beginning of the fatless period than later. In this case the first observation on the urine was made on February 26, 1896, when the animal had been for four days on centrifugalized milk. The urea increased irregularly, but on the whole gradually, from 4.86 gm. on Feb. 26 to 29.47 gm. on April 11 and 20.32 gm. on April 13. During this time there was an irregular but relatively slight increase in the P_2O_5, so that

the ratios increased from 1 to 10.94 at the beginning to 1 to 32.40 at the end of the period.

We thus have records of three animals fed on nearly fatless milk, in all of which the excretion of P_2O_5 is very distinctly lower than in animals fed on milk containing a natural proportion of fat; in two of these cases the study of the urine was begun sufficiently early in the experiment to permit of the detection of a marked fall in the excretion of P_2O_5 as a result of the continuation of the fatless diet.* In the milk on which these animals were fed, which has been shown to be remarkably constant in composition, it is presumable that the P_2O_5, present chiefly in salts of Ca and Mg, bore a fairly constant relation to the N present in the proteids of the milk. The N of the urine and the P_2O_5 of the urine are admitted to be ultimately derived from the food ingested. How then are we to explain the fact here noted that the P_2O_5 excretion did not keep pace with the N excretion? The P_2O_5 unaccounted for must be assumed either to have been retained in the body, probably as the Ca and Mg salts of growing bone, or to have been lost through the fæces owing to defective absorption. As the latter explanation was thought to be more probable it was determined to study the effect of adding a considerable amount of fat in the form of suet to the usual quantity of fatless milk consumed by pig. 9. One hundred grammes of suet, therefore, were given daily with the milk. The feeding remained the same in all other respects. The effect on the urine was immediate and striking. The absolute amount of the P_2O_5 increased rapidly to a somewhat higher average than any previously observed in the course of this experiment, in spite of the fact that the absolute urea excretion fell distinctly. The result was an immediate return to the ratios normally observed in pigs on a mixed diet. This may be looked upon as strong evidence in favor of the view that the low P_2O_5 excre-

*The fall in the absolute amount of P_2O_5 in pig 1 may perhaps be explained by the fact that the quantity of milk received by him was relatively small. Pigs 8 and 9 show no absolute fall in P_2O_5. Pig 8 was given an unlimited quantity of milk, pig 9 was on a fixed amount, but this was considerably larger than in the case of pig 1.

tion observed in these experiments is the result of defective absorption of phosphates from the intestine. This assumption is further reinforced by a study of the excretion of the salts through the fæces. In pig 1 we find that the ash of the fæces took on an increase from 16.96% of the solid matter of the fæces on Dec. 24, 1893, to 47.48% on Jan. 13, 1894, to 49.92% on April 10 and 41.33% on Oct. 5. The first figure is within the normal limits on a mixed diet; the remaining percentages are all distinctly high. In pig 8 the figures representing the percentages of the salts are all very high, ranging as they do from 42.53% to 70.89%. In this case the percentages are only a little higher at the end of the period of observation than at the beginning, but it should be remembered that the analyses were not begun until the animal had been nearly 8 weeks on a fatless diet. In pig 9 again it is found that the percentages of the salts run high throughout (from 36.16% to 54.95%), but in this case the observations were begun two weeks after the beginning of the experiment, and there is a very evident gradual increase in the percentages of the salts lost by way of the bowel. It is interesting to note in this connection that on the addition of fat to the diet of pig 9 there was a prompt fall in the percentage of salts passed with the fæces. Of course the mere fluctuations in the fæcal fat would account for a considerable fluctuation in the percentage of ash, but not for the marked differences here noted. It is further noticeable that the percentage of P_2O_5 and of Ca of the total salts shows a slight progressive increase in both pig 8 and pig 9, which of course corresponds to a considerably greater increase in the absolute P_2O_5 and CaO which is lost through the fæces. The results obtained make it clear that the presence of fat in the diet exerted an important influence on the absorption of salts, especially those of P_2O_5 from the intestine, in the animals experimented upon. They show, at least in the case of pigs, that fat starvation entails a marked diminution in the absorption and in the renal excretion of P_2O_5. Whether the same relationship holds good generally in the higher mammals cannot be stated, but it seems likely that it is very generally operative as a physiological principle. I have several times noted the presence of an unusually low P_2O_5

content of the human urine when the quantity of fat ingested was much restricted. This suggests that the same relationship applies in the human subject. At present I know of no satisfactory explanation of this interdependence.

Whether the deficient supply of salts to the organism is a factor in producing the fat atrophy here described must be regarded as exceedingly doubtful. It is much more likely that its chief effect is to produce the striking retardation in the development of the skeleton which was noted in pigs 1 and 8. Of interest in this connection is the fact that the analysis of the solids of the femur gave essentially the same percentages of P_2O_5, CaO, and of ash as the femur of a normal pig of the same age but of much greater skeletal development.

There are other features of interest in the comparison of the analyses made of the various organs of pig 1 with those made upon the organs of a normal animal, but I shall here mention only a few of the more striking results. Thus it is notable that the percentage of fat in the femur of pig 1 was only .48% of the solids, whilst that in the femur of a normal pig was 2.85% or almost six times as much. The percentage of fat in the femur of pig 2, the control animal, on a preponderating carbohydrate diet, was 8% of the solids or sixteen times as much as in pig 1. These figures well illustrate the general impoverishment of the body in fat. In the case of the liver the reduction is much less pronounced. The ether extract from the liver of pig 1 was 8.9% of the solids, from pig 2 it was 12.2%, and from a normal animal 12%. The preservation of so considerable an amount of fat in the liver is in accord with the experiments of Noël Paton, which showed that in pigeons subjected to a fast of four days there was no reduction in the normal amount of fatty acids in the liver, although any excess of fat stored in the liver was rapidly reduced to normal.*

* Probably the object of this storing of fatty acids (as suggested by Paton) is to provide the necessary material for the synthesis of lecithin by the combination of phosphorus with fatty acids. The nucleins of the cells are constantly breaking down, and the store of fatty acids seems necessary to hold the P_2O_5 which results from this katabolism. See D. Noël Paton, On the Relationship of the Liver to Fats, *The Journal of Physiology*, xix (1896), 167.

It is also noteworthy that notwithstanding the very great reduction of fat in the body, the lecithins of the brain, which are phosphorized fats, were preserved in their normal proportions. This is shown by the following figures:

	Pig 1.	Pig 3 (normal).
Lecithins of grey matter,	2.6%	2.9%
Lecithins of white matter,	5.2%	4.3%

The chief features of interest in the chemical study of the blood were the fact that in pig 1 the serum globulin was markedly reduced in proportion to the serum albumin, and that the urea was considerably below the normal percentage.

The most obvious, and perhaps the most interesting, consequence of the foregoing experiments in fat starvation is the universal serous or gelatinous change in the subcutaneous and other fat depositories. From what has already been said of the microscopical and chemical characters of this altered adipose tissue in pigs 1 and 8, it seems reasonable to interpret as follows the process which leads to extreme changes like those noted in pig 1. After a few months of fat starvation the fat cells shrink somewhat, but their contents continue to consist wholly of fat. Next the contents of some of the cells break up into larger and smaller fat globules and a material between these globules which is not fat and stains feebly with hæmatoxylin and basic aniline dyes. This change becomes universal, the cells diminish very much in size, the cell membrane grows irregular in outline owing to the diminution in the volume of the cell body, and the nucleus is no longer applied to the cell membrane but may occupy any position in the cell. In time the much shrunken contents contain no fat whatever and consist merely of a small amount of homogeneous material which stains faintly with hæmatoxylin, and in some cases of a small amount of finely granular matter near the nucleus. Inasmuch as the transformed layer of adipose tissue maintains almost the volume of an abundant normal subcutaneous fat layer, in spite of the shrinkage of the cells to one-third or one-sixth of their normal size, and inasmuch as there is nowhere any evidence of cell multiplication, it is reasonable to suppose that the volume of

the transformed layer is maintained in part by the presence of a newly formed intercellular substance. Of this the separation of the shrunken cells and the separation of the bundles of elastic fibres into their individual fibres may perhaps be regarded as evidence.

The appearances noted in the epicardial and perirenal fat of pig 8, where, probably owing to more successful preservation, an abundant intercellular substance was present, confirm the opinion here expressed in reference to the nature of the conditions in pig 1.

The histological changes which occur in atrophic adipose tissue have been made the subject of careful study by Flemming,* Czajewicz,† Schmidt‡ and others. Flemming in his classical studies of fat atrophy showed that when fat tissue undergoes atrophy, as seen in experimental starvation or in wasting diseases, the atrophic fat cells do not all undergo the same changes. In many cases three distinct types of atrophy could be distinguished. The commonest form of atrophy is that characterized by a general and evenly distributed reduction in the size of the fat cells, the cells undergoing no alteration either in contour or in the character of their contents. This condition is known as simple fat atrophy. It is often overlooked in the human subject. It is a usual accompaniment of a variety of nutritive and other disorders characterized by wasting.

Another type of fat atrophy is distinguished by cell proliferation. Here the small laterally placed nucleus of the fat cell, together with the small quantity of protoplasm by which it is surrounded, undergoes cell division. As the proliferative process advances the fat content of the cell becomes transformed and disappears. The newly proliferated cells were at one time thought to resemble leucocytes, and Waldeyer maintained that they actually were wandering cells. There are, however, several facts which are inconsistent with this

* Flemming's studies are by far the most important contributions relating to the subject of fat atrophy. The following are his chief writings upon this subject: W. Flemming, *Centralbl. f. d. med. Wissenschaft*, 1870, No. 31; *Arch. f. mikr. Anat.*, vii (1870), 32 and 328; Virchow's *Archiv*, lii (1871), 568 and lvi (1872), 146; *Arch. f. mikr. Anat.*, xii (1876), 391.

† F. Czajewicz, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1866, 289.

‡ H. Schmidt, Virchow's *Archiv*, cxxviii (1892), 58.

view—the implication of isolated fat cells, the large size attained by the proliferated cells, their branching form and the manner in which the cell bodies sometimes remain connected with one another by slender trabeculæ after nuclear division has occurred. This process, which is especially apt to occur in fat cells where rapid and extreme wasting has occurred, is described by Flemming as atrophic proliferation (atrophische Wucherung).

The process of atrophic proliferation differs strikingly from the third form of atrophy described by Flemming, namely, serous atrophy (seröse Atrophie) or gelatinous atrophy (gallertige Atrophie) in which the content of the fat cells is a homogeneous or finely granular material which contains no fat, and in which the transformed and atrophied fat cells are separated by a serous or gelatinous substance which is thought to be of an albuminous nature. This condition of serous atrophy has been observed by Flemming, Schmidt and others in atrophic fat in association with the condition of atrophic proliferation, but it often occurs independently. It has been found in the human subject in states of prolonged inanition, such as phthisis and cancer. The alteration is usually confined to the epicardial, perirenal and omental fat, but is sometimes found elsewhere.

The structural alterations which were observed in pigs 1 and 8 show no definite indications of the process of atrophic proliferation as it occurs in the atrophy due to the action of iodine, in that which follows the mechanical constriction of nutrient vessels or in the atrophy which occurs in rheumatic nodules.* Very few of the atrophic fat cells contain more than one nucleus, and there is nowhere seen the cell division which characterizes proliferative atrophy. Although the intercellular connective tissue is more cellular than that seen in normal adult fat, this seems to be due partly to arrest of development, partly to approximation of atrophied cells.

On the other hand, the appearances noted in pigs 1 and 8 bear so

* I have seen a beautiful example of fully developed atrophic proliferation in a preparation from a small mass of human omental fat which, as the result of an operation, had suffered constriction from the remaining omental fat during several days. I am indebted to Dr. Shelby, of the Loomis Laboratory, for an opportunity to study this material.

close a resemblance to the condition of so-called serous atrophy as it occurs in the human subject that there can be no doubt as to the identity of the processes. The propriety of the name "serous atrophy," implying as it does a replacement of the normal contents of the cell by serum, may well be questioned in view of the observations recorded in this paper. I have, however, continued to use this term as the one generally adopted to designate the same condition in human beings. Comparing the appearances seen in the gelatinous fat atrophy observed in the epicardial fat in wasting diseases, with those which I have produced experimentally, it is found that in both conditions there is a marked shrinkage of all the fat cells, with transformation of their contents, into the same serous or gelatinous material that constitutes the abundant intercellular substance. In both conditions the absence of fat is demonstrable by means of osmic acid. In neither state is the vascularity of the atrophic fat so great as in normal fat. Certain points of difference may, however, be recognized. In the atrophic fat cells of experimental origin only one nucleus is seen in each cell. Very rarely two nuclei, surrounded by a small amount of protoplasm, were demonstrated in the same cell; a larger number was not observed. In preparations made from atrophic pericardial fat, on the contrary, a considerable number of cells appear in which nuclear multiplication has occurred in the atrophic fat cell.

It may be stated, without fuller description of the histology of the proliferative process, that in such cases of serous fat atrophy as have come to my notice in the human subject, the process of atrophic proliferation has co-existed with the process of serous atrophy. In these cases the evidences of cell proliferation have existed in only a small proportion of the atrophic fat cells; the serous atrophy has been the preponderating, but not the exclusive process. In the atrophic fat which develops as the consequence of prolonged fat starvation we may regard the serous form of fat atrophy as the sole lesion. It seems probable, from a consideration of the literature relating to the atrophy of fat, that the experimental serous fat atrophy observed in the animals which form the subject of this paper is the type of serous fat atrophy that has been described.

It seems probable, on experimental grounds, that in pigs at least, serous fat atrophy can be produced only by prolonged deprivation of fat; the removal of carbohydrates, of proteids or of salts giving rise merely to simple atrophy, or to a limited and disseminated serous atrophy. It seems not unlikely that the advanced process of serous fat atrophy observed in the human subject, in the course of chronic tuberculosis and other wasting diseases, depends, similarly, on the presence of a very low percentage of fat in the blood.

Another question which suggests itself is whether the full explanation of the serous atrophy of fat starvation lies in the deprivation of fat to which the organism is subjected. Moreover, does fat starvation under all circumstances entail serous atrophy of fat tissues if the privation be continued long enough? In the case of pig 1 it will be remembered that the supply of proteid and carbohydrate food, though considerable, was restricted. May it not be that simple starvation is in part responsible for the observed changes? The fact that the same kind of changes, though less extreme, was going on in the skin of pig 8 while he was being given an unlimited supply of fatless milk of the same composition as that given pig 1, indicates that general starvation does not play an essential role in producing the beginnings of the condition.

The findings in the case of pig 2 make it clear that the removal of fat from the milk to the extent practised in these experiments does not necessarily induce the serous atrophy. In this animal, as already explained, there was even less fat and proteid ingested than in pigs 1 and 8, but an enormous quantity of carbohydrate food was given. As is well known, mammals have the power of converting carbohydrates into fat, probably chiefly in the liver and through the intermediate stage of glycogen formation, and it is in the highest degree probable that this is the explanation of the absence of the serous atrophy in pig 2. It is to be noted here that though the carbohydrate excess prevented the occurrence of serous atrophy, it was not capable of obviating the gradual absorption and simple atrophy of the subcutaneous fat.

There remains to be noted a collateral feature of considerable in-

terest, namely, the chronic degeneration of the kidney which was observed in pig 1. The explanation of this which appears most probable is that the degeneration resulted from the long-continued excessive activity of the renal epithelium in the excretion of the N-containing constituents of the urine, which, owing to the highly nitrogenous diet, were excreted in pronounced excess. That such exaggerated physiological activity on the part of the renal epithelium may give rise to degeneration was conclusively shown, it is believed for the first time, by the following experiment:

The left or larger kidney was removed from a healthy dog whose daily urea excretion ranged from 10 to 15 grammes on a sufficient diet. When the animal recovered from the operation he was placed on an excessive meat diet, with the result that the excretion of urea was increased to 30 or 40 grammes daily. The remaining kidney was thus compelled to do several times the work which it would ordinarily be required to do. The experiment was continued for one year, during which time the animal was kept in confinement but under otherwise hygienic conditions. He seemed well most of the time, but had occasional vomiting seizures and periods of loss of appetite. He had no symptoms of any acute infectious disease. Autopsy showed that the remaining kidney was perhaps slightly larger than normal; the cortex was rather pale and the markings indistinct, the epithelium of the secreting tubes was the seat of distinct granular degeneration, and there were occasional thin cortical wedges of young connective tissue. For several weeks before the animal was killed the urine had contained a trace of albumin but no casts. The kidney first removed was normal.

Another indication that the changes found in the kidneys of pig 1 were the result of their prolonged overwork is the fact that the kidneys of pig 2, whose nitrogenous food was much more limited than that of pig 1, showed no degenerative change. During the last days of pig 1 there were fever and some pneumonic consolidation, and though it is not likely that the renal changes were the result of infection, its occurrence necessitates caution in the interpretation of the degeneration.

CONCLUSIONS.

The following inferences may be made from the foregoing study:

1. The lesions resulting from fat starvation, at least in the case of pigs, do not resemble or even suggest those of rickets.

2. Prolonged fat starvation leads to the entire disappearance of fat from the adipose tissues. The form of fat atrophy observed as the result of experimental fat starvation corresponds to the serous fat atrophy described by Flemming, and is essentially the same type of fat atrophy as that found in the epicardial and perirenal fat in the human subject as the result of wasting disease.

3. The lecithins of the brain and the fat of the liver are not materially reduced by fat starvation.

4. Fat starvation does not lead to advanced serous fat atrophy of the subcutaneous fat if the animal be given a large excess of carbohydrate food or a considerable excess of the carbohydrate and proteid constituents of milk.

5. Fat starvation causes a very imperfect absorption of the salts of P_2O_5 from the intestine.

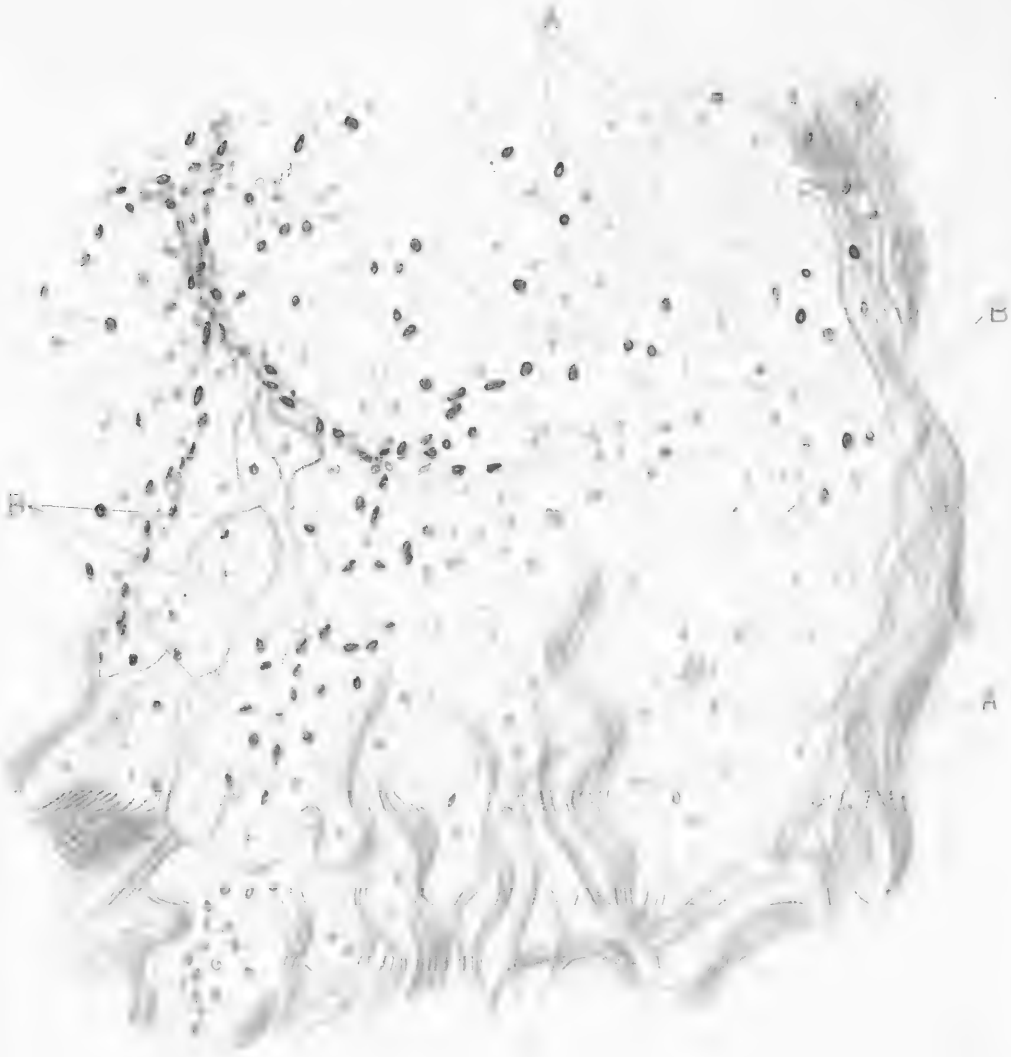
DESCRIPTION OF PLATES XXIII AND XXIV.

PLATE XXIII.

Shows size and appearance of normal fat cells from subcutaneous fat from back of neck of a normal pig aged one year. Drawn with camera lucida. Ocular 4, objective C (Zeiss).

PLATE XXIV.

Fat from back of neck of Pig 1, one year after commencement of diet consisting of milk from which fat was largely removed. A, fat cells which have undergone serous atrophy. Osmic acid does not stain the contents of these cells. The intercellular matrix is not represented. B, capillaries, some of them with blind ends. C, bundles of elastic fibres. Drawn with camera lucida. Ocular 4, objective C, Zeiss.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STUDIES ON TRICHINOSIS, WITH ESPECIAL REFERENCE TO THE INCREASE OF THE EOSINOPHILIC CELLS IN THE BLOOD AND MUSCLE, THE ORIGIN OF THESE CELLS AND THEIR DIAGNOSTIC IMPORTANCE.

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PLATES XXV-XXVII.

INTRODUCTION.

Since the discovery by Zenker of the Trichina spiralis as a lethal agent in the human being, and the placing of the disease trichinosis or trichiniasis on a firm clinical basis, the subject has been studied with great care from many points of view. Indeed, the fact that with the study of this affection—its ætiology, symptomatology, pathology and prophylaxis—such names as those of Virchow (70), Leuckart (40), Cohnheim (13) and Zenker (74) are intimately connected, vouches for the completeness of our knowledge regarding many of its features.

Modern methods, however, have given rise to new possibilities in the line of more minute histological and pathological study, and it is to this fact and to the unusually favorable conditions surrounding the present cases that this contribution owes its origin.

The Trichina spiralis was discovered in the human cadaver in 1835 by Owen (53); it possessed, however, mainly zoological interest until, by the work of Zenker in 1860, a strong stimulus was given to the study of the trichina and of trichinosis. The various German epidemics (13, 57, 42) occurring since then have furnished abundant material for investigators, and as a result of their observations and experiments it is now known that the main source of infection in man is incompletely cooked pork, which in a certain percentage of instances contains encapsulated trichinæ in the muscle fibres.

After trichinotic flesh has been eaten the capsules are dissolved off by the gastric juice; the worms pass into the small intestine, where

they reach their sexual maturity; copulation then takes place, and on the 6th or 7th day after the ingestion of the meat the first embryos appear. From the intestine the embryos pass to the muscular tissues. Some, especially Leuckart (40), regard this passage as taking place by an active boring of the embryos through the loose connective tissue; others (14, 2, 21) think that the distribution occurs chiefly through the vascular and lymphatic systems, a view which the recent work of Cerfontaine (14) and Askanazy (2) seems to favor. These observers assert that the female worms, after penetrating the villi, bring forth their young directly in the lymph spaces. After the embryos have reached the muscles, changes take place in them which will be described a little later.

CLINICAL HISTORY OF CASE I.

In the following case which was under observation in the medical clinic of the Johns Hopkins Hospital from March 3 until May 13, 1896, the blood was examined daily, with a determination of the number of leucocytes per cubic millimetre and a differential count of the various forms; frequent examinations of the urine and quantitative determinations of the uric acid, urea and total nitrogen were made, while on two different occasions small pieces of muscle were removed and subjected to careful microscopic examination.

Robert T., aged 23 years, an Englishman by birth, was admitted to the hospital on March 3, complaining of general pains. His past history was of no especial interest excepting that for the last three months he had been living the life of a tramp, with continual exposure to the weather and with insufficient nourishment.

Six weeks before entering the hospital he began to complain of general pains, as he expressed it, in the joints, bones and muscles. For the last two weeks he had been much worse, scarcely able to move about. On entrance he complained of a feeling of fulness in his head. No diarrhœa; no cough; no œdema at any time.

Physical examination showed the patient to be rather pale; tongue thickly coated; the temperature at time of admission was 102° F.; pulse 104; respirations 24. Examination of the thorax was negative, except for a soft systolic murmur heard all over the cardiac area; very faintly heard in the mid-axilla. Patient complained of general soreness in the limbs. Pressure on the muscles almost everywhere caused great pain, particularly in the right biceps. There was no tenderness apparent in the bones or in the skin. Spleen not palpable; no rose spots. Urine:

color normal; acid; 1028; albumin, trace; sediment, slight, showing mucous cylindroids, hyaline and granular casts. Diazo-reaction was not present.

Examination of the blood on March 5 showed 17,000 leucocytes with 37 per cent of eosinophiles. The absence of rose spots and of any palpable enlargement of the spleen, as well as the presence of a leucocytosis and the extraordinary increase in eosinophilic cells, rendered the diagnosis of typhoid fever, which was at first considered, improbable. The extreme muscular tenderness on pressure and on voluntary or passive motion, and the irregular temperature, suggested rather a myositis, and a diagnosis of trichinosis was made.

On further questioning the patient stated that six or seven weeks previously he began to have vague pains and heavy feelings in the muscles of his legs, and that he had repeatedly eaten raw or incompletely cooked pork during this period.

To confirm the diagnosis, on March 12 a small piece of muscle was removed under cocaine from the right biceps, where the pain was greatest at that time. A teased specimen of this showed, on microscopic examination, trichinæ, the majority actively motile, a few apparently non-motile; none were encapsulated, but many were surrounded by a finely granular material. During the remainder of the patient's stay in the hospital the symptoms gradually abated; the temperature, which had run a very irregular course, slowly descended to the normal point, and the sensitiveness of the muscles to pressure decreased. Œdema of the ankles appeared on two occasions in April after walking about the ward for several days, but both times quickly disappeared after rest in bed.

On May 13 the patient was discharged, the following note being made: "For from two to three weeks there has been no pain in the muscles; the stiffness has wholly disappeared and the patient feels perfectly well in every way, excepting that violent exertion is not readily borne."

Thus it will be seen that in many respects our case showed the classical picture of trichinosis. The pain in the muscles, aggravated by pressure or motion, the similarity of the early symptoms to those of typhoid fever, the œdema of the ankles after exercise, and the rather irregular fever, were characteristic. The absence of any history of gastro-intestinal symptoms, usually so marked, was probably due partly to the fact that this was not a case of extreme severity and partly to the incoherence of the patient on admission.

THE BLOOD.

Although it was known to the older pathologists that there were various kinds of white cells in the blood, it is only since the demonstration by Ehrlich (19) of their varied reactions to different dyes that we have possessed an adequate means of differentiating the various forms and of studying their relative proportions, one to another, in health and disease.

Morphologically, and by means of these tinctorial reactions, the leucocytes may, as is well known, be divided into five classes:

(1) Small mononuclear cells.
(2) Large mononuclear cells.
(3) Transitional cells.
(4) Polymorphonuclear neutrophilic cells.
(5) Eosinophilic cells.

In the first two divisions—the small and large mononuclear cells (the classification being determined by the fact that they are smaller or larger, respectively, than the ordinary polymorphonuclear neutrophiles) —the protoplasm shows no evidence of specific granulation and the nucleus is round or oval.

In the cells of the third division—the transitional—the nucleus becomes notched or indented and the protoplasm shows sometimes a slight tendency to neutrophilic granulation.

In the last two varieties, the polymorphonuclear neutrophiles and the eosinophiles, the granulations are well marked, in the former being fine and neutrophilic, in the latter coarse, highly refractive and acidophilic. The nucleus of the former is polymorphous; of the latter usually also polymorphous, although sometimes round or oval.

Many of the English school, notably Kanthack and Hardy (36), and Gulland (24), regard the so-called neutrophilic granulations as slightly acidophilic, and for this reason call the cells containing them the finely granular oxyphiles or acidophiles or eosinophiles. In the present work, however, the name polymorphonuclear neutrophiles (or cells with the ε granulation) will be used in describing them.

The percentage of these various forms in the blood differs quite markedly within normal limits. The usual proportions in the adult as given by Kanthack are:

Small mononuclears	15-25 per cent.
Large mononuclears and transitional forms	6 "
Polymorphonuclear neutrophiles	70-75 "
Eosinophiles	1-5 "

Concerning the proportion of the cells about which we are especially interested, the eosinophiles, various observers have given slightly different figures; Ehrlich (19*c*) regards it as from 2-4 per cent usually, although sometimes normally reaching 10 per cent; Hayem (31) as 7 per cent; Gabritschewsky (25), 1-3 per cent; Müller and Rieder (47*b*), 1-4 per cent; Canon (15), 1-3 per cent; v. Limbeck (41), 2-8 per cent; Uskov (quoted in 58), 6 per cent; Zappert (73), 0.67-11 per cent; while Weichselbaum (64) and Gollasch (26) each gives 5 per cent, including the transitional forms.

In this study the average number of leucocytes per cmm. has been taken to be 6500, of which 74 per cent (4810) should be polymorphonuclear neutrophiles, 18 per cent (1170) small mononuclears, 6 per cent (390) large mononuclears and transitionals, 2 per cent (130) eosinophiles.

Ever since the discovery of the specific cell granulations the eosinophilic cells have attracted the attention of many investigators who have studied the changes in the blood, especially in reference to the increase or decrease of this particular form in various pathological conditions. Much greater diagnostic significance than would seem to be warranted has, in many instances, been ascribed to this increase or decrease. Nevertheless, in certain diseases, chief among which are bronchial asthma and spleno-myelogenous leukæmia, a constant increase in these cells has been noted.

In the latter disease, as noted by Schwarze (60), Spilling (61), and Müller and Rieder (47*b*), the increase is more absolute than relative, owing to the enormous increase in the total number of leucocytes per cmm. In bronchial asthma an increase, both relative and absolute, has been described by Gollasch (26), Fink (20) and Gabritschewsky (25). In certain affections of the skin, especially pemphigus, prurigo, psoriasis and chronic eczema, a marked increase in eosinophiles has been noted by Canon (15) and Neusser (51). Kotschetkoff (37) describes an increase in scarlet fever, and Zappert (73) and Müller and Rieder (47*b*) in anchylostomiasis. Besides these, many isolated reports of an increase are to be found, although in many cases this has been too slight to be of significance. An increased percentage of eosinophiles has been noted in syphilis (4), malaria (1, 17, 27*a*), diseases of the genital organs and bones (51), mental diseases (51), during childhood (29), and after the injection of tuberculin (27*b*, 69, 5).

The largest percentages of eosinophiles hitherto reported have been 22.40 per cent in bronchial asthma (25), 33.02 per cent and 29.28 per cent in two cases of pemphigus, 27.9 per cent in anchylostomiasis (73), and 21.1 per cent in anterior poliomyelitis (47*b*). In a case of chronic eczema attending the Johns Hopkins Hospital, three different counts of the blood gave 22.6, 24 and 22.6 per cent of eosinophiles.

In the present case an examination of the blood on March 5 showed that the eosinophiles constituted 37 per cent of all the leucocytes—so striking an increase above the normal that from that time until the patient's discharge the blood was examined daily, and a determination of the number of leucocytes per cubic millimetre and a differential count of the various forms were made.

As a routine proceeding in making the differential estimate 500 leucocytes were counted daily, the stain employed being Ehrlich's triple stain (orange G., acid fuchsin and methylene green). In the case of the larger percentages an additional 500 stained in a different mixture (hæmatoxylin and eosin, methylene blue and eosin, Chenzinsky's solution, Huber's or Weichselbaum's modification of Ehrlich's triacid stain, Neusser's modification of the triple stain) were counted as a control.

The cover-glass preparations from which these counts were made were fixed either by Ehrlich's method of heating at 110° C. for from 1 to 1½ hours, or by Nikiforov's method of immersion in a mixture of equal parts of absolute alcohol and ether for two hours. The Zeiss mechanical stage was employed in studying the preparations.

In determining the number of leucocytes per cubic millimetre the Thoma-Zeiss hæmocytometer was used, the diluting agent being either Toison's mixture or an $\frac{1}{3}$ per cent solution of acetic acid.

The red blood corpuscles, counted at various intervals during the disease, showed a gradual increase from 4,200,000 per cubic millimetre, three days after the patient's admission, to 4,900,000 per cubic millimetre on the day of his discharge.

When we consider the changes which the blood underwent during the two months of the patient's stay (see Table I, p. 322, and the Chart (Plate XXV)), some very interesting facts are brought out.

From their original percentage of 37, the eosinophiles gradually fell to between 10 and 15 per cent, where they remained for about two weeks, the number of leucocytes per cubic millimetre during this period ranging between 15,000 and 20,000. On March 26 the percentage of eosinophiles began to rise, this rise continuing steadily and gradually until April 23, when it reached 68.2 per cent; this means that *more than two-thirds of all the white blood cells were eosinophiles.* During this time an absolute increase in the leucocytes was also noted, the number per cubic millimetre averaging 27,500 between April 8 and April 22. From April 23 the percentage of eosinophiles decreased, and there was also a decrease in the total number of leucocytes, and on May 12, the day previous to the patient's discharge, 16.8 per cent and 11,000 were found respectively.

During the whole time the character of the eosinophiles was apparently perfectly normal—their nuclei were almost exclusively polymorphous, and they were slightly larger than the polymorphonuclear neutrophiles (Plate XXVI). As was to be expected, the percentage of these latter (always quite normal in character) varied inversely with that of the eosinophiles, reaching as high as 80.4 per cent and sinking as low as 6.6 per cent. The striking nature of this inverse relationship may be better appreciated if one studies the accompanying table and the chart (Plate XXV).

The small mononuclear leucocytes, scanty at first, soon increased to between 10 and 20 per cent, where they subsequently remained; in fact the percentage of both small and large mononuclear and transitional forms showed very slight fluctuations.

It is interesting to note that after about two weeks the percentage of small mononuclears kept fairly constant, being about that normally found in the blood. When we consider the very marked leucocytosis, this indicates that the total number of small mononuclears was much increased. When and how this increase takes place, however, must be left open to conjecture.

A few typical myelocytes were seen, averaging less than 0.3 per cent, probably no more than one would expect with so marked a leucocytosis.

TABLE I. CASE I.—SHOWING TOTAL NUMBER AND PERCENTAGE OF VARIOUS KINDS OF LEUCOCYTES.

Date.	Leucocytes per cmm.	Percentage of Various Forms of Leucocytes.				Number of the Various Forms per cmm.			
		P. M. Neut.	S. Monos.	L. M. and T.	Eos.	P. M. Neut.	S. Monos.	L. M. and T.	Eos.
March 5....	17000	55.0	3.0	5.0	37.0	9350	510	850	6300
6....	16500	50.0	5.0	7.0	38.0	8250	825	1120	6120
7....	17500	53.0	3.0	7.4	36.4	9275	525	1200	6300
8....	25300	60.9	1.9	5.9	31.4	15000	500	1500	7750
9....	22300	70.8	2.0	4.5	22.7	15300	440	900	5060
10....	16500	74.7	3.1	8.0	15.1	12000	480	1280	2400
11....	21167	75.1	2.8	2.4	18.6	15800	630	500	3700
12....	17500	77.8	4.4	3.0	15.1	13600	770	510	2625
13....	18833	77.6	2.1	3.7	16.6	13860	380	710	3000
14....	13800	74.6	4.3	4.0	14.9	10300	580	560	2100
15....	13750	74.0	7.6	3.2	13.6	10300	1000	420	1720
16....	13250	78.7	5.3	2.1	13.8	10400	680	270	1700
17....	16000	73.2	11.3	1.9	13.5	11700	1770	310	2160
18....	17750	77.4	10.9	1.7	10.0	13800	1970	300	1775
19....	20400	71.1	13.1	2.3	13.1	14200	2600	460	2600
20....	15700	76.5	10.1	2.2	11.2	12100	1570	340	1760
21....	16800	72.9	13.2	1.8	12.1	12400	1210	320	2040
22....	14000	79.2	10.8	1.7	8.3	11200	1540	240	1130
23....	16800	73.6	10.4	1.6	13.5	12400	1700	270	2200
24....	19600	74.6	11.2	1.8	11.2	14800	2200	360	2200
25....	17600	80.4	8.9	1.6	8.7	14400	1600	290	1560
26....	24000	69.2	12.7	2.0	15.4	16800	3120	480	3700
27....	20300	69.9	11.2	2.2	16.0	14000	2240	440	3200
28....	24100	68.3	10.1	2.4	18.9	16300	2400	580	4560
29....	20700	69.4	5.7	1.4	22.9	14000	1180	300	4600

TABLE I.—*Continued.*

Date.	Leucocytes per cmm.	Percentage of Various Forms of Leucocytes.				Number of the Various Forms per cmm.			
		P. M. Neut.	S. Monos.	L. M. and T.	Eos.	P. M. Neut.	S. Monos.	L. M. and T.	Eos.
March 30....	22300	60.7	9.6	3.0	26.0	13400	2200	670	5720
31....	22200	57.4	11.2	3.2	27.8	12600	2500	680	6160
April 1....	24300	60.7	7.9	3.4	27.8	14400	1920	840	6720
2....	23800	59.3	11.6	3.0	25.0	14100	2760	710	5950
3....	25200	53.3	13.3	2.8	30.0	13300	3300	700	7500
4....	23400	49.9	13.3	3.0	32.8	11700	3200	710	7590
5....	24300	48.8	13.2	4.0	33.8	10100	3150	960	7160
6....	24700	51.2	14.0	3.6	31.6	12700	3500	650	7900
7....	25100	48.4	14.5	2.5	33.7	12100	3600	625	8600
8....	29600	45.2	11.6	4.0	38.8	13500	3450	1180	11600
9....	28900	45.0	13.3	3.2	38.3	13000	3850	930	11000
10....	24500	45.8	12.2	3.8	38.0	11300	3100	900	9300
11....	29800	42.2	14.0	4.0	39.8	12600	4150	1180	11900
12....	28000	44.0	14.8	2.5	38.7	12300	4150	700	10800
13....	24200	42.0	11.8	1.4	44.2	10000	2880	340	10700
14....	33300	38.6	13.2	3.8	44.0	12800	4400	1300	14700
15....	28100	34.8	19.6	1.6	44.0	8600	5550	450	12300
16....	25400	30.4	16.8	3.0	49.6	7600	4200	750	12400
17....	27000	31.8	13.2	3.4	51.2	8600	3500	920	13700
18....	35700	27.2	14.4	3.8	54.6	9700	5140	360	19500
19....	29000	26.6	12.0	3.6	58.2	7700	3480	1040	16900
20....	19200	22.2	12.4	4.2	61.0	4200	2350	800	11600
21....	23000	16.6	16.0	3.8	63.6	3800	3680	870	14600
22....	26600	10.4	18.8	7.4	63.4	2700	4900	1950	16500
23....	17700	6.6	19.6	5.2	68.2	1170	3400	880	11070

TABLE I.—*Continued.*

Date.	Leucocytes per cmm.	Percentage of Various Forms of Leucocytes.				Number of the Various Forms per cmm.			
		P. M. Neut.	S. Monos.	L. M. and T.	Eos.	P. M. Neut.	S. Monos.	L. M. and T.	Eos.
April 24....	18800	7.2	23.4	4.4	64.8	1370	4400	840	12200
25....	15600	8.8	19.2	7.2	64.8	1370	2990	1020	10400
26....	17000	12.4	14.2	6.0	67.4	2100	2410	1020	11400
27....	12000	20.0	8.6	7.4	64.0	2400	1030	890	7700
28....	11100	18.2	17.6	7.2	57.0	2020	1950	800	6330
29....	12800	19.4	19.4	6.8	54.4	2470	2480	870	6990
30....	13200	20.8	17.2	8.2	53.8	2750	2270	1080	7020
May 1....	11000	21.8	16.0	12.0	50.2	2400	1760	1320	5520
2....	8900	27.4	17.8	8.4	46.4	2440	1580	740	5160
3....	10700	36.8	15.0	6.8	41.4	3940	1600	710	4430
4....	10700	34.8	20.8	6.4	38.0	3720	2120	680	4070
5....	11000	45.6	19.0	6.6	28.6	5020	2090	710	3150
6...	10300	41.0	20.2	4.8	33.6	4220	2080	490	3460
7....	12300	50.8	20.2	6.0	23.0	6250	2480	740	2830
8....	11500	54.0	18.0	4.0	24.0	6210	2070	460	2760
9....	11500	59.8	17.6	6.2	16.4	6850	2020	710	1890
10....	15700	51.0	12.8	5.6	20.4	7990	2010	880	3200
11....	13500	62.4	14.2	4.8	18.8	8420	1920	650	3540
12....	11000	64.0	14.4	4.8	16.8	7040	1580	530	1850

If one estimate the total number of these various forms per cubic millimetre (see Table I, p. 322) by multiplying the percentage of the individual variety by the total number of leucocytes per cmm., it will be found that, for a period of two weeks, the polymorphonuclear neutrophiles were *absolutely decreased* in amount—a very striking fact when one considers the marked leucocytosis. This tends to show even more clearly the remarkable relationship which exists between the neutrophiles and the eosinophiles, the latter cells being greatly increased in number during the same period.

Plate XXVI shows a typical microscopic field of the blood of Case I. The three most striking points furnished by the study of the blood are:

(1) The remarkable increase in the number of the eosinophiles, which amounted to 68.2 per cent of all the leucocytes present, 35 per cent more than has ever hitherto been reported.

(2) The coincident diminution in the number of the neutrophiles, these cells and the eosinophiles being at all times in inverse proportion.

(3) The marked leucocytosis.

The presence of such great quantities of eosinophiles in the blood in this case is a point of especial interest. If further observation shows this change to be characteristic we shall be furnished with a symptom of the greatest diagnostic value in this disease, and one which, perhaps, may help to clear up some of the cases which are regarded *intra vitam* as rheumatic in nature, the true diagnosis being revealed only years afterwards upon the autopsy table.

As an association has for a long time been noted between the eosinophiles and the Charcot-Leyden crystals, occurring so commonly in the spleen and marrow in leukæmia (52*b*) and in the sputum in bronchial asthma (20, 45, 46, 54), it was regarded as interesting to see whether from the blood in this case, containing as it did such large quantities of eosinophiles, the crystals might be obtained.

For this purpose blood was withdrawn on nine occasions, placed sometimes in sterilized, at other times in unsterilized vessels, some kept in the thermostat at 37.5° C., others at room temperature; but,

although repeated and careful microscopical examinations were made at frequent intervals during a period of several weeks, in none of the specimens were any Charcot-Leyden crystals to be found.

This completely harmonizes with the conclusions reached by H. F. Müller (47*a*), who carried on investigations of a like nature upon the contents of fresh pemphigus vesicles, which also contain great quantities of eosinophilic cells. Thus it seems highly probable that the Charcot-Leyden crystals are, at least, not *direct* crystallization products of the eosinophiles and that something besides the presence of these cells is necessary for their formation.

THE URINE.

The urine, as has been mentioned, showed a trace of albumin, hyaline and finely granular casts and a small number of pus cells. No sugar was found. Ehrlich's diazo-reaction was never present.

A systematic quantitative study of the nitrogenous elements of the urine was carried on chiefly in connection with the ideas of Horbaczewski (30). This observer, as is well known, believes that the uric acid excreted by the animal organism is derived from the destruction of nuclein-holding material and mainly from the destruction of leucocytes.

This view is based on the following assumptions:

(1) That outside the body we may obtain uric acid from nuclein-holding substances.

(2) That in leukæmia and leucocytosis, where there is a greater destruction of leucocytes, there is a distinct increase in the uric acid excretion.

(3) That in many pathological conditions where nuclein-holding substances are destroyed, as in fevers, conditions of inanition, phosphorus-poisoning and cachexia, the increase in uric acid is also noted.

(4) That in the experimental leucopenias, produced by quinine and atropine, a diminution in the uric acid excretion may be shown.

The quantitative work was carried on in this case mainly with a view to control the second of the above statements, that is that a parallelism exists between the extent of the leucocytosis and the

amount of uric acid excreted, a parallelism which has, however, been denied by Mareš (48), who regards the uric acid as a by-product in leucocyte formation.

To determine this question in the present case the total quantity of uric acid excreted during the 24 hours was determined on 23 different occasions; on four of these days the total nitrogen and on four others the total urea per 24 hours was also estimated.

The uric acid was determined by the Hopkins method—precipitation of the uric acid from 100 cc. of the urine by saturation with ammonium chloride, filtration, solution of the uric acid, reprecipitation with strong hydrochloric acid and final titration with potassium permanganate. The total nitrogen was estimated by the Kjeldahl method, *i. e.* conversion of all the nitrogen into ammonia and titration with a standard solution of oxalic acid. The urea was determined by oxidation with sodium hypobromite, and measurement of the nitrogen given off.

TABLE II. CASE I.—URINE CHART.

Date.	Total Quantity per 24 Hours of			Ratio of	
	Uric Acid.	Urea.	Total Nitrogen.	Uric Acid : Urea.	Nitrogen in Uric Acid : Total Nitrogen.
March 15....	.2423 gram.				
16....	.2092 "				
17....	.5722 "				
18....	.7800 "				
19....	.8180 "				
20....	.3869 "				
21....	.4053 "				
22....	.2088 "				
23....	.7935 "				
24....	.3119 "				
25....	.4579 "				
26....	.4542 "				
27....	.1975 "				
28....	.5882 "				
April 3....	.3685 "		7.9497 grams.		1 : 64.7
4....	.2829 "		7.2300 "		1 : 76.6
5....	.2753 "		4.6725 "		1 : 50.9
6....	.3619 "		4.8027 "		1 : 39.8
7....	.3971 "				
24....	.2905 "	10.79 grams.		1 : 37.1	
28....	.3716 "	19.22 "		1 : 51.7	
29....	.4893 "	29.11 "		1 : 59.4	
30....	.2946 "	17.21 "		1 : 58.4	

By examination of Table II it will be seen that on no occasion did the total uric acid excretion, the ratio of uric acid to urea excretion, or the ratio of nitrogen in the uric acid to the total nitrogen in the urine, rise to any extent above the limits usually regarded as normal.

The normal quantity of uric acid and the normal ratio of this to the urea is given somewhat differently by different authors; by Hammarsten (32) as 0.7 gramme and 1:50-70; by Halliburton (33), 0.55 gramme and 1:60; by Vierordt (71), 0.67 gramme and 1:45; and by v. Jaksch (35), 0.21 gramme and 1:60 respectively. Hammarsten (32) gives the normal ratio of nitrogen excreted in the form of uric acid to the total nitrogen as 1:69.3.

Thus the results obtained in this case show such a slight, if in fact any, relative or absolute increase in the uric acid excretion that they speak against the views of Horbaczewski; for if this author's results are universally correct we should expect a decided increase—absolute or at least relative—in the uric acid excretion in a case where the leucocytes were as markedly increased as they were in our case. It is an interesting fact, however, that here the eosinophiles and not the neutrophiles, as is usually the case in a marked leucocytosis, were the elements especially increased.

THE MUSCLES.

The changes in the muscles in trichinosis have, of course, been repeatedly and carefully studied; in the early sixties by Virchow (70), Zenker (74), Fiedler (21), Cohnheim (13) and many others; more recently by Soudakewitch (62), Lewin (43), Geisse (28) and Finger (22). All of these observers describe the presence of the parasites in the primitive muscle bundles and the granular degeneration which takes place about them; they further speak of the great proliferation of the muscle nuclei, so that in some places, especially in the region of the invaded fibre, there may be great masses or clumps of nuclei situated in undifferentiated sarcoplasm. In other places swelling and degeneration of the nuclei have been described. The method of formation of the capsule or cyst wall which surrounds the parasite after it has been in the muscle a certain length of time is still undecided. Many ideas have been held. It has been suggested that it arises from a thickening of the sarcolemma (70*a*, *b*, *d*, 6); from the interfascicular connective tissue (16); from the finely granular degenerative detritus (74*a*); or partly

from secretion from the worm itself and partly from inflammatory changes in the surrounding connective tissue (7). The more modern observers have described further changes not mentioned by the older pathologists.

Finger (22) notes the presence of great numbers of small round cells about the vessels and capsules and in the interfibrillar connective tissue, and the splitting up of the contents of the sarcolemma sheaths into small irregular bits, in most of which the cross striation has been lost.

Soudakewitch (62), besides this breaking up of the muscle fibre into little bits, notes also the presence of great numbers of leucocytes which he regards as phagocytes of the degenerated fibres. This phagocytic action is also shared by elements which he regards as muscle-cells, and which arise by the separation from the rest of the muscle fibre of the nucleus with some of the muscle substance about it. These structures appear to maintain for a while an independent existence, but soon undergo destruction.

Lewin (43) has devoted himself more especially to the cell changes. He describes the proliferation and great hypertrophy of the muscle nuclei in the fibres containing trichinæ, with a degeneration of many of these nuclei as shown by the affinity of the nucleoli for the acid stain; the splitting up of the muscle fibre into separate cells; the formation of separate cells of the kind regarded by Soudakewitch as phagocytic muscle cells, and the local œdema of many of the fibres.

The portions of muscle removed in this case, both of which came from the right biceps, were hardened immediately, the specimen of March 12 in alcohol, that taken two weeks later in bichloride of mercury. On microscopic examination many of the above described changes are to be noted (Plate XXVII).

In the first specimen the reaction is especially marked in a few foci; in some places the fibre containing the parasite has become converted into a finely granular, faintly staining material, containing many large, swollen nuclei—the proliferated muscle nuclei—the nucleoli here taking the usual deep blue hæmatoxylin stain. The sarcolemma shows slight thickening, but there is no very marked heaping up of cells outside of it except in a very few places. Elsewhere absolutely no change in the muscle fibre is to be seen, excepting a small layer not thicker than the diameter of a red blood corpuscle, surrounding the parasite, which is very small; in fact, the whole picture points conclusively to a very recent infection.

In many of the fibres not containing parasites marked nuclear proliferation is seen; in some this is found in conjunction with granular degeneration of the muscle substance, in others with no apparent changes; about the nuclei, especially of the former, a distinct but rather small vacuole may be made out, a sign, probably, of lowered vitality of the nucleus.

A peculiar fragmentation of the muscle is sometimes to be noted. At times it takes place in the direction of the long axis of the fibres, which break up into separate fibrillæ or bunches of fibrillæ; at other times it occurs in an irregular manner. In a few places a peculiarly interesting picture is observed, the fragmentation being transverse and the axis of the proliferated nuclei running also in a transverse direction, thus splitting the muscle into distinct disks. Whether in this latter mode of fragmentation the division had occurred at the membrane of Krause or the plane of Hensen could not be made out, owing to the obscuring of the field by the great number of proliferated muscle nuclei.

Throughout the specimens there are to be seen cells whose protoplasm shows a marked affinity for the eosin stain. These cells contain one nucleus or sometimes two or three, generally of rather large size. The fact that some of these elements are still in part connected with the muscle fibre, and that in the protoplasm of some there are still evidences of striation, points at once to their origin by the separation of a certain amount of muscle substance, containing one or several nuclei from the primitive muscle bundle.

These cells, together with bits of degenerating muscle, some pale and granular, others taking on a deep eosin stain, but both showing a total or almost complete loss of striation, and many pale and swollen muscle nuclei, are seen in quite large numbers in certain scattered foci of degeneration present in the muscle.

What is of especial interest, however, is that all through the muscle, between and within the fibres, but especially in those areas made up largely of cells and debris, many polymorphonuclear cells are seen; some of these are ordinary pus cells, neutrophiles; others show large red refractive acidophilic granules in the cell body, *i. e.* they are

eosinophiles; in some again the protoplasm, though finely granular, shows a distinct affinity for the eosin stain; in other words, the character of the cells appears to be half-way between that of the neutrophiles and that of the eosinophiles.

These cells would appear to be phagocytic in nature, for they are especially abundant in the more degenerated areas, and in many places are seen in little bays or inlets or lakes in the degenerating muscle, as if gnawing it away. It was thought at first that perhaps these cells might ingest bodily the little rod-shaped bits or fragments which are found especially at the ends of the degenerating muscle fibres and which with the hæmatoxylin and eosin stain show considerable resemblance to the granules in the eosinophilic cells; but staining with other double stains and with the triple stain showed that this supposition is hardly tenable, since the staining properties of the two are markedly different. The degenerated substance if taken up by the leucocytes must undergo a rapid disintegration within the cell body or possibly may be taken up in a soluble form.

In the second specimen, taken two weeks later, more advanced stages of the same processes are seen. The trichinæ are larger, and in some cases two or three parasites are found in the same cyst; about the cysts there is a marked increase in the number of nuclei; in some cases true giant cells are formed, a number of nuclei lying in a mass of undifferentiated protoplasm, probably sarcoplasm, while throughout the whole specimen the nuclear proliferation is more marked. The transverse splitting of the fibres into disks and the transverse proliferation of the contained nuclei is more marked than in the preceding specimen, as well as the longitudinal and irregular breaking up of the fibres.

The vacuoles about the proliferating and degenerating nuclei are more marked; the muscle cells, mentioned above, show less affinity for the eosin stain and in some cases give evidence of granular degeneration; besides the slight thickening of the sarcolemma there is no distinct capsule about the parasites. Of the polymorphonuclear cells, the eosinophiles are much more common, the neutrophiles much less numerous; the former are present everywhere, but are found especially

in the scattered foci of more marked degeneration and about the ends of degenerating fibres (Plate XXVII).

That these were typical eosinophiles was shown not only by the character and size of their granules, but also by the fact that they showed the typical staining properties with acid fuchsin, orange G., picrate of ammonium and other acid stains, and the Biondi-Haidenhain triple stain for tissues as well as with the ordinary hæmatoxylin and eosin stain (Plate XXVII).

In the interfibrillary connective tissue there was a slight proliferation of the cells, while the changes in the interfascicular connective tissue were barely evident. In the blood-vessels in the latter, counts of the white blood cells showed that the neutrophiles and eosinophiles were present in about the same ratio (never differing more than 3 or 4 per cent) as in the blood count of the corresponding day, thus suggesting that the eosinophiles were not attracted to the part to a greater extent than the neutrophiles.

The presence of such great numbers of eosinophiles was so striking that two specimens of trichinotic muscle, found in the Pathological Museum of the Johns Hopkins Hospital, were examined to see whether like changes were present.

In the first, a case of carcinoma mammæ in which trichinæ were found in the pectoral muscle, the capsules and parasites were for the most part calcified, although some showed fatty and fibrous change; in the muscle no especial changes were noted. This, of course, was a case of old standing, discovered by chance, probably years after the original infection.

The second specimen, fortunately, was from a case of acute trichinosis which had caused the death of the patient, a New York butcher, six weeks after the onset of the disease. Here the muscle is studded with cysts, each containing from one to six trichinæ, while about these, and in fact all through the muscle, great quantities of nuclei are seen, about many of which there are distinct vacuoles; in some cases the nucleoli take the eosin stain. The muscle cells, mentioned before, here show still further degeneration; the protoplasm is granular and takes a yellowish color, while the nuclei stain very slightly with hæma-

toxylin. But what is most interesting is that here again numbers of typical eosinophiles are to be seen scattered everywhere throughout the specimen, but congregated more especially in the areas of more marked degeneration, side by side with the degenerating bits of muscle and the muscle cells.

Thus these two cases, both acute and both studied shortly after the infection, besides the marked changes in the muscles, characterized by the great proliferation of nuclei, the formation of muscle cells and their subsequent degeneration, the formation of distinct vacuoles about the proliferating and degenerating muscle nuclei, and the granular degeneration of many of the fibres, show an interesting and hitherto undescribed condition, namely, the presence of large numbers of eosinophilic cells. This fact, coupled with their coincident marked increase in the circulating blood, suggests one way at least in which these cells may arise in the body.

THE INCREASE IN THE EOSINOPHILIC CELLS.

Concerning the nature of the eosinophilic granules and the origin of the cells containing them, many widely divergent views have been held, some founded entirely upon staining reactions, others built on a somewhat firmer basis, all more or less hypothetical, some very far-fetched and bizarre.

Until the introduction of the Ehrlich stains, these coarse, refractive granules were regarded as fat droplets by Wharton Jones (34), who first described them in 1846, by Förster (23) and Biesiadecki (8), who saw them in the blood in leukæmia, and by Neumann (52), Bizzozero (9), Budge (10) and Mosler (49), who noted them in the bone-marrow in the same disease. The cells containing these granules are the coarsely granular leucocytes of Max Schultze (63).

The work of Ehrlich (19) and Schwarze (60) showed, however, that these large refractive granules are not fat droplets, but bodies possessing a chemical nature not clearly understood. Ehrlich first demonstrated the affinity of this granulation for the acid coloring matters. These eosinophilic granules, as they are now generally termed, are regarded by him and his school as a sort of nutritive reserve, built up by the cell for its own use or for excretion. Thus, according to Schwarze (60), they resemble somewhat the aleuron granules or crystalloids of plants

in being the products of a specific cell activity, and are probably not proteid in nature. Zappert (73) and Przewoski (56), however, insist that these granules are of the nature of proteid, the latter showing that their solubilities and chemical reactions correspond in many respects to this form of matter.

Many investigators, among whom may be mentioned Pouchet (55), Bannwarth (11) and Przewoski (56), basing their arguments upon the similarity in the reaction of these granules towards the acid aniline dyes, have regarded them as related to or identical with hæmoglobin, though Ehrlich (19) and Schwarze (60) deny this and show that there are quite marked tinctorial differences between the eosinophilic granules and hæmoglobin.

The demonstration of the presence of iron in these granules by Barker (12) is of especial interest in this connection.

Sherrington (65), who states that he demonstrated phosphorus in them by the Lilienfeld-Monti (44) method, regards them as a form of nucleo-albumin, while Kanthack and Hardy (36) consider them to be secretory products containing a zymogen, giving rise to alexines or other substances of great activity in phagocytosis and in conditions of inflammation and intoxication.

In all the above views the granules are regarded as paraplastic in nature. Gulland (24), on the other hand, holds that they are plasmatic; or, in other words, that they represent an altered condition of the microsomes and are connected by a mitoma.

As a consequence of this diversity of opinion regarding the nature of the granules, the mode of origin of the cells is still an unsolved problem. The view most generally held is that they are derived from the polymorphonuclear neutrophiles by a kind of ripening process, a view which was advanced by Max Schultze (63) and which is now held by Ehrlich (19), Gulland (24), Zappert (73) and many others. Müller and Rieder (47*b*, *c*) and van der Stricht (66) think that, besides this mode of formation, some eosinophiles are derived by mitosis from pre-existing cells of the same kind. Rénaut (59), Denys (18) and Arnold (3) describe amitotic division of these elements.

Max Schultze (63) and Müller and Rieder (47*b*, *c*) hold that this change takes place mainly in the circulating blood; according to Ehrlich (19) the bone-marrow is the only place where this transition occurs; while van der Stricht (66) and Zappert (73) think that blood and hæmatopoietic organs both play a part in this process.

Kanthack and Hardy (36) regard these elements as wandering cells whose home is normally the connective tissue; Saxer (68) thinks that all

leucocytes are derived from pre-existing primary wandering cells, while Gollasch (26), Neusser (51) and Weiss (72) believe that they are derived directly from the tissue elements.

Of those who think they can trace a relationship between eosinophiles and the red blood corpuscles, Przewoski (56) regards the eosinophiles as incompletely developed erythrocytes, *i. e.* derived from hæmatoblasts, while Sacharoff (67) believes that they are formed through the engulfing by leucocytes of nucleoli cast out by hæmatoblasts (nucleated red corpuscles) at the time when the latter are converted into erythrocytes.

The study of the blood and the tissues of our cases of trichinosis furnishes us with important evidence bearing upon the origin of the eosinophilic cells. The constant inverse relationship existing between eosinophiles and neutrophiles, the increase of the former being invariably coupled with a coincident decrease of the latter, so that the neutrophiles on some occasions were absolutely subnormal in number notwithstanding the marked leucocytosis, as well as the similarity in size and the character of the nuclei of the two varieties of cells, both point strongly toward some very close connection between the two forms. Indeed, the striking and constant inverse relationship between the neutrophiles and eosinophiles would appear to be strong evidence in favor of the view that the latter cells arise, in some instances at least, by direct transformation of the former. There is much also to suggest that this transformation may have occurred here in the muscles. The presence in the first piece of muscle of many neutrophiles, together with many typical eosinophiles and cells which might be regarded as transitional forms between the two, and the finding in the second specimen two weeks later of a greatly increased number of eosinophiles, harmonize with this idea, especially when we consider that *on both of these occasions the ratio of the neutrophiles to the eosinophiles in the peripheral blood and in the vessels of the interfascicular connective tissue was between four and five to one,* a very much larger ratio than that which exists between these cells in the muscle.

How such a transformation might take place one can only conjecture. One may imagine that the trichinæ on reaching the muscle act as a strong irritant or poison, giving rise to many tissue changes, and probably producing either primarily or secondarily chemotactic

substances which attract large numbers of leucocytes to the muscles, causing a marked leucocytosis, especially in the more degenerated portions. In these regions the polymorphonuclear neutrophiles wandering out of the vessels act as phagocytes upon the degenerating or degenerated bits of muscle. It is in these phagocytes apparently that the change in the character of the granules takes place.

By this it is not meant that the eosinophilic granules are bits of ingested material, but that they are elaborated by the cell itself. May not this result perhaps be due to the ingestion of some material originating from the degeneration of the muscle fibres, which may bring about some essential change in the character and chemismus of the cell?

Finally, we may well suppose that these cells wander or are swept back into the general circulation, giving us the marked increase in eosinophiles. Such a theory would not seem unreasonable, particularly when we consider the amount of muscle tissue in the body. The fact that practically no atypical neutrophiles or eosinophiles, *i. e.* evidences of transition, were seen in the blood itself speaks against the probability that such a change may have taken place in the circulation. Again, these small polymorphonuclear cells are certainly very different in appearance from the large, often mononuclear eosinophiles which we find in the blood and know to come from the marrow in leukæmia.

It is interesting to note that during the course of this investigation an article appeared by Kischensky (39), in which he comes to the same conclusion regarding the origin of the eosinophiles in the sputum of asthmatics, in which these cells are present in very large quantities, namely, that they are derived from neutrophiles.

After the completion of the above work and the publication of a preliminary note thereon in the *Johns Hopkins Hospital Bulletin* of April, 1897, a second case entered the Johns Hopkins Hospital, which in almost all respects tended to confirm the results obtained in the preceding investigations.

CLINICAL HISTORY OF CASE II.

The patient, E. B., a sailor, age 29, white, of German nationality, was admitted, April 15, 1897, complaining of fever, chills, muscular pains and weakness.

His past history shows that he has always enjoyed very good health; he gives no history of rheumatism nor of œdema, and has been only a moderate drinker. During his voyages his ship has often stopped at Cuba, but the sailors have never been allowed to go on shore, and he said there had been no sickness whatever amongst the crew. It was later found, however, that two members had malarial fever of the æstivo-autumnal type.

The present sickness dates from the first week in April. The onset was quite insidious; loss of appetite and general muscular weakness were noted, with occasional heavy painful sensations in the epigastrium. Headache set in for the first time on Friday, April 9, and continued until admission, the pain being especially marked in the frontal region. There had been considerable pain in the muscles of the back and especially in the calves of the legs since April 11. The bowels were regular until April 15, when the patient had two fluid stools, but no tenesmus. On April 10 the patient noticed a rather sudden œdema of the eyelids and face, causing him much discomfort and some pain in opening and closing the lids. He had had pain in the facial muscles during mastication for the last three days, while on deep inspiration there had been a slight pain through the chest. There had been no epistaxis. On board the ship the patient ate mostly salted meats and had frequently eaten raw sausage, though never raw ham. He had had shaking chills at times.

The physical examination showed heart and lungs negative; good color of lips and mucous membranes; gums normal; slight pain and stiffness in the masseters on opening and closing the mouth, and slight puffiness of the eyelids. Pressure on larynx is slightly painful, but no pain on pressure of calf muscles. No pain in arms and thighs. Spleen is just palpable; abdomen is a little full but not tender, and has upon it one or two spots somewhat suggestive of rose spots. There is no glandular enlargement; no tenderness or nodes on the long bones.

The temperature at first was of an intermittent type, rising each evening to between 103° and 105.5° F. and sinking to normal by the following morning. After April 24 the temperature continued normal until the time of the patient's discharge.

The stools, which continued to be of fluid consistence for some time, on frequent examinations showed no parasites or ova of parasites.

The urine was negative. The examination of the blood was made very frequently and carefully on many occasions, but no malarial parasites were ever obtained, nor were they obtained from the spleen, which was aspirated for this purpose.

The blood, however, showed other marked changes, chief among which was a great increase of the eosinophile cells, reaching 42.8 per cent on one occasion, accompanied by a moderate leucocytosis. This very marked eosinophilia, coupled with other points in the clinical history of the case, but mainly the eosinophilic increase, suggested the possibility that the case might be one of trichinosis. On April 28 a small piece of muscle from the calf was removed, and the diagnosis was confirmed by the demonstration in it of young non-encapsulated trichinæ. These were not present in such large numbers as in Case I, as might have been expected from the fact that the present patient complained of much less pain and in general showed less marked symptoms.

The subsequent history of the case was, as in the previous instance, a gradual cessation of all the symptoms, a disappearance of the muscular pain and a slow decrease in the percentage of eosinophiles, there being 17.6 per cent when he left the hospital, still a considerable increase above the normal.

THE BLOOD.

The blood in this case showed in many respects the same features as in the previous one (Table III). Both eosinophilia and leucocytosis, however, were less marked, as one would naturally have expected, since we were here dealing with a much less severe infection, if one may judge from the much milder clinical symptoms and the discovery of fewer parasites in the portion of muscle removed. As the patient was under observation a much shorter time, the table is not so complete as that belonging to Case I. The eosinophiles, which on admission made up 42.8 per cent of the total leucocytes of the blood, gradually sank to between 14 and 17 per cent, while in the corresponding period the polymorphonuclear neutrophiles rose from 43.1 to between 58 and 67 per cent (see Table III).

The leucocytosis varied between 6,000 and 13,000 during the patient's stay in the hospital, averaging about 10,000 and not varying markedly. The small mononuclears showed at first a tendency to be much decreased, as in the previous case; but, as was also seen before, showed later only slight fluctuations.

TABLE III. CASE II.—BLOOD CHART.

Date.	Leucocytes per cmm.	Percentage of the Various Forms of Leucocytes.				Total Number of Various Forms per cmm.			
		P. M. Neut.	L. M. and T.	S. Monos.	Eos.	P. M. Neut.	S. Monos.	L. M. and T.	Eos.
April 15....	13000	43.1	6.5	1.4	42.8	5600	180	840	5560
16....	....	47.7	4.7	4.5	39.1	....	....	....	....
17....	....	52.0	2.8	7.6	37.6	....	....	....	....
18....	8000	....	....	....	....	....	....	....	....
19....	....	57.2	2.8	8.0	32.0	....	....	....	....
20....	8900	55.2	3.8	11.2	31.8	4900	1000	340	2830
21....	8700	....	....	....	....	....	....	....	....
22....	10700	58.0	2.7	11.7	27.7	6200	1250	290	2960
23....	6000	56.2	2.0	15.2	26.2	3370	910	120	1570
24....	11000	60.4	4.0	13.2	22.4	6640	1450	440	2460
25....	11000	57.2	4.2	18.6	20.0	6290	2050	460	2200
26....	9600	60.7	5.3	12.3	21.7	5830	1180	510	2080
27....	11300	63.0	5.0	17.0	14.7	7120	1920	560	1660
28....	12700	62.7	4.0	17.0	16.3	7960	2160	510	2070
29....	12000	67.0	3.25	15.25	14.0	8040	1830	390	1680
May 1....	10700	58.3	4.0	22.0	15.7	6240	2350	430	1680
3....	13000	64.3	4.0	16.0	15.7	8360	2080	520	2040
5....	12000	64.7	4.0	16.0	15.3	7760	1920	480	1840
7....	9300	62.8	4.0	16.4	16.8	6140	1530	370	1560
10....	10700	60.4	2.8	16.4	20.0	6460	1750	300	2140
12....	11000	60.4	3.3	16.6	19.6	6640	1820	360	2160
14....	12000	58.8	2.8	18.8	19.6	7060	2260	340	2350
17....	9000	58.8	3.6	20.0	17.6	5290	1800	320	1580

When one considers the morphological structure of the different white blood cells one is again struck by the fact that mononuclear eosinophiles are almost entirely wanting, the nucleus of these cells being almost exclusively polymorphous and resembling in every way that of the neutrophiles. Here also, as in Case I, extremely few myelocytes were seen (averaging 0.03 per cent), and but few forms which could be regarded as transitional forms.

The number of red blood corpuscles per cmm. varied between 5,000,000 and 5,120,000.

THE MUSCLE.

The changes in the muscle in this case, although some of the features described before were lacking, resemble in many respects those seen in the first case. They were, however, by no means so well marked.

There is proliferation of the muscle nuclei, especially in the fibres, which show by their loss of staining properties a beginning degeneration. The number of degenerating fibres, however, is small, and these are entirely confined to the neighborhood of the trichinæ. The nuclei of the fibre containing the trichina are pale and greatly swollen, the nucleoli large and deeply staining; a few karyokinetic figures are seen.

Surrounding the large typically curved trichina in its muscle fibre, which shows granular degeneration, we have many small round cells, "muscle cells," muscle nuclei, polymorphonuclear cells (mostly neutrophilic, a few eosinophilic) and bits of muscles containing many swollen proliferated nuclei.

The striation of some of the degenerated fibres is entirely lacking; in others the peculiar granular degeneration noted in the first case is seen.

There are some eosinophiles scattered throughout the specimen, though not comparable in number to those noted in the first case; here more of the polymorphonuclear cells are neutrophiles.

There is no evidence of transition from neutrophiles to eosinophiles in this specimen. In fact the whole picture is one of a much less violent infection than in the first case. In all probability the trichinæ

had been in the muscle fibre a longer time than in the first case, since when the bit of muscle was removed the number of eosinophiles per cmm. had fallen to a comparatively low point, nor did they rise again, a point which suggested, as did the clinical history, that the infection had almost run its course.

Recently, since the preceding pages were placed in the hands of the editor, a third case of trichinosis has come under our observation, which tends still further to establish our belief in the conclusions arrived at from the study of the first two cases.

In this, as in the second case, the discovery of a largely increased number of eosinophiles in the peripheral circulation led to the idea that the case was one of trichinosis, and an examination of an excised bit of muscle again proved the correctness of this idea.

Case III.—The patient, J. Y., was admitted to the hospital, December 21, 1897, complaining of headache, pain in the side, chilly sensations and swelling of the feet. Six months previously he had been treated for typhoid fever in the hospital and still gave the Widal reaction. Seven years previously he had had an attack of acute articular rheumatism.

The patient lived with his German employer and frequently partook of sausages and other forms of swine meat, often insufficiently cooked.

For five weeks he had been feeling unwell, complaining of frontal headaches and coughing spells; there had been no herpes, nose-bleed nor shaking chills. Five days before entering the hospital the patient had had an acute attack of diarrhœa, having 5 to 8 thin, green stools daily, associated with severe griping pains in the abdomen. Two days after the commencement of the diarrhœa he had noticed that his legs, feet, hands and arms seemed numb and cold. The joints were stiff and the limbs swollen and his attention was called by his employer to his swollen eyelids.

On admission the physical examination showed an absence of rose spots, spleen not palpable, puffiness of the eyelids, great tenderness of the muscles of the arms and of the calf, with swelling of the former and a bluish mottling of the skin of the limbs.

The temperature reached 103° for the first few days and then gradually fell to normal; shortly after this the pain in the limbs and the intestinal symptoms decreased, and on January 16, 1898, the patient was discharged, apparently quite well except for slight soreness of the right leg.

The blood (Table IV) on admission showed 45 per cent of eosinophiles, and this, from the study of the two previous cases, led to the diagnosis of trichinosis and the subsequent confirmation of that diagnosis by the removal of a portion of muscle and the discovery of trichinæ therein.

TABLE IV. CASE III.—BLOOD CHART.

Date.	Number per cmm.		Percentage of			
	Red blood corpuscles.	Leucocytes.	Polymorphonuclear neutrophiles.	Large mononuclears and transitional.	Small mononuclears.	Eosinophiles.
Dec. 22..	....	....	48.4	4.7	1.5	45.4
23..	....	17,000	52.7	3.6	3.1	40.4
27..	4,700,000	15,300	42.4	4.0	5.6	45.0
Jan. 3..	4,300,000	12,000	42.2	4.2	4.6	49.0
7..	....	14,700	31.6	4.4	19.0	44.6
10..	4,546,000	....	32.4	3.0	21.8	42.8
14..	....	13,700	35.2	4.0	23.4	37.2
22..	....	9,000	45.5	2.0	17.7	34.7

During the stay of the patient in the hospital the proportion of eosinophiles varied very slightly, reaching 49 per cent on January 3 and sinking to 34.7 per cent on January 22. As in the other cases, the nuclei of practically all these cells were polymorphous; yet, as before, no forms were seen in the blood suggesting transitional stages between neutrophiles and eosinophiles. At first there was a smaller percentage than normal of the small mononuclears, which, however, increased later up to practically the normal, a condition which was noted in both the preceding cases. On one occasion, and only one, two typical myelocytes were seen. The leucocytosis was not marked, being 17,000 on December 23 and 9,000 on January 22 (see Table IV).

As in the preceding cases, it was impossible to determine how long the eosinophilia lasted, as in all three cases the patients left town and were lost from observation.

The stools, although examined repeatedly, showed no intestinal trichinæ.

The piece of muscle removed during the first week of the patient's presence in the hospital showed in almost all respects the same changes noted in the two previous cases, except in this case the specimens of the parasite found in the muscle were younger, smaller and less abundant than in the first case. In the muscle fibre in which the trichinæ were found there was a complete loss of striation and a tendency to take on a faint bluish stain. In these fibres the proliferated and greatly swollen muscle nuclei with their deeply staining nucleoli were seen arranged circularly about the trichinæ lying in the finely granular, faintly staining, degenerated muscular substance.

Near the infested fibres were found degenerating areas made up of disintegrating bits of muscle fibre, muscle nuclei, small round cells, and polymorphonuclear cells, a great many of whose granules were eosinophilic. These eosinophilic cells were especially prevalent about the bits of degenerating muscle and were certainly as numerous as in the first case.

There was the peculiar granular degeneration of the muscle fibres noted before, and the proliferation of the muscle nuclei in the neighborhood of the parasite.

The trichinæ were young, only slightly curled up and of course non-encapsulated.

In severity, clinically, this case lies midway between the first and second cases, and this position is borne out by the number of parasites found in the muscle excised, by the leucocytosis and by the grade of eosinophilia.

SUMMARY.

To summarize, we have been able to demonstrate:

(1) In a case of acute trichinosis an extensive leucocytosis, with great absolute and relative increase in the number of eosinophilic cells in the blood, associated with a coincident decrease in the quantity of neutrophilic elements.

(2) From the examination of specimens of muscle removed during life, besides the peculiar degenerations of the muscle, a longitudinal

splitting of some of the fibres; a remarkable transverse splitting of others; a great proliferation of nuclei, about many of which vacuoles are seen; and large numbers of polymorphonuclear eosinophilic cells, which are especially prevalent in the more degenerated areas.

(3) In a second case (after death), besides similar changes in the muscle, large numbers of eosinophiles throughout the infested portion.

(4) In two other cases, during life, a great increase of the eosinophilic cells in the blood, with a coincident decrease of the polymorphonuclear neutrophiles, associated with leucocytosis, though of less extent than in the first case.

(5) In pieces of muscle removed in these last two cases changes in most respects similar to those cited in the first case, but of less degree.

(6) The similar character of the nuclei of the eosinophiles and the neutrophiles both in the blood and in the muscle, and the presence in the first case of certain cells which might be regarded as forms transitional between neutrophiles and eosinophiles, suggesting the possibility that the increase in the latter elements may, in these instances, take place in the muscles by direct transition from the neutrophiles.

CONCLUSIONS.

From these observations it is fair to conclude:

1st. That there is a marked increase in the percentage of eosinophilic cells in the blood in trichinosis.

2nd. That this increase may be used as a diagnostic sign in this disease.

3rd. That this disease in its sporadic form is more common than has hitherto been supposed, as shown by the discovery of the three cases above described, within a comparatively short period, at the Johns Hopkins Hospital.

4th. That a systematic examination of the blood should be undertaken in cases with indefinite intestinal, muscular or articular symptoms, in the hope that in some, at least, of the hitherto doubtful cases a diagnosis may be reached.

In conclusion I wish to express to Professor William Osler my sincere appreciation of his kindness in giving me the opportunity of

studying these cases, and to Dr. William Sidney Thayer my hearty thanks for his help during the course of this investigation.

DESCRIPTION OF PLATES XXV–XXVII.

PLATE XXV.

Chart showing graphically the total number and percentages of the various kinds of leucocytes.

PLATE XXVI.

From the blood of Case I, showing a typical field, containing six eosinophiles, with polymorphous nuclei, one polymorphonuclear neutrophile, one large mononuclear leucocyte and many erythrocytes.

PLATE XXVII.

Showing degenerating area in muscle in neighborhood of trichina, demonstrating beginning loss of striation in two fibres, complete loss in a third, and in the rest of the specimen, muscle nuclei, bits of degenerating muscle tissue and many polymorphonuclear eosinophiles. Haidenhain-Biondi triple stain.

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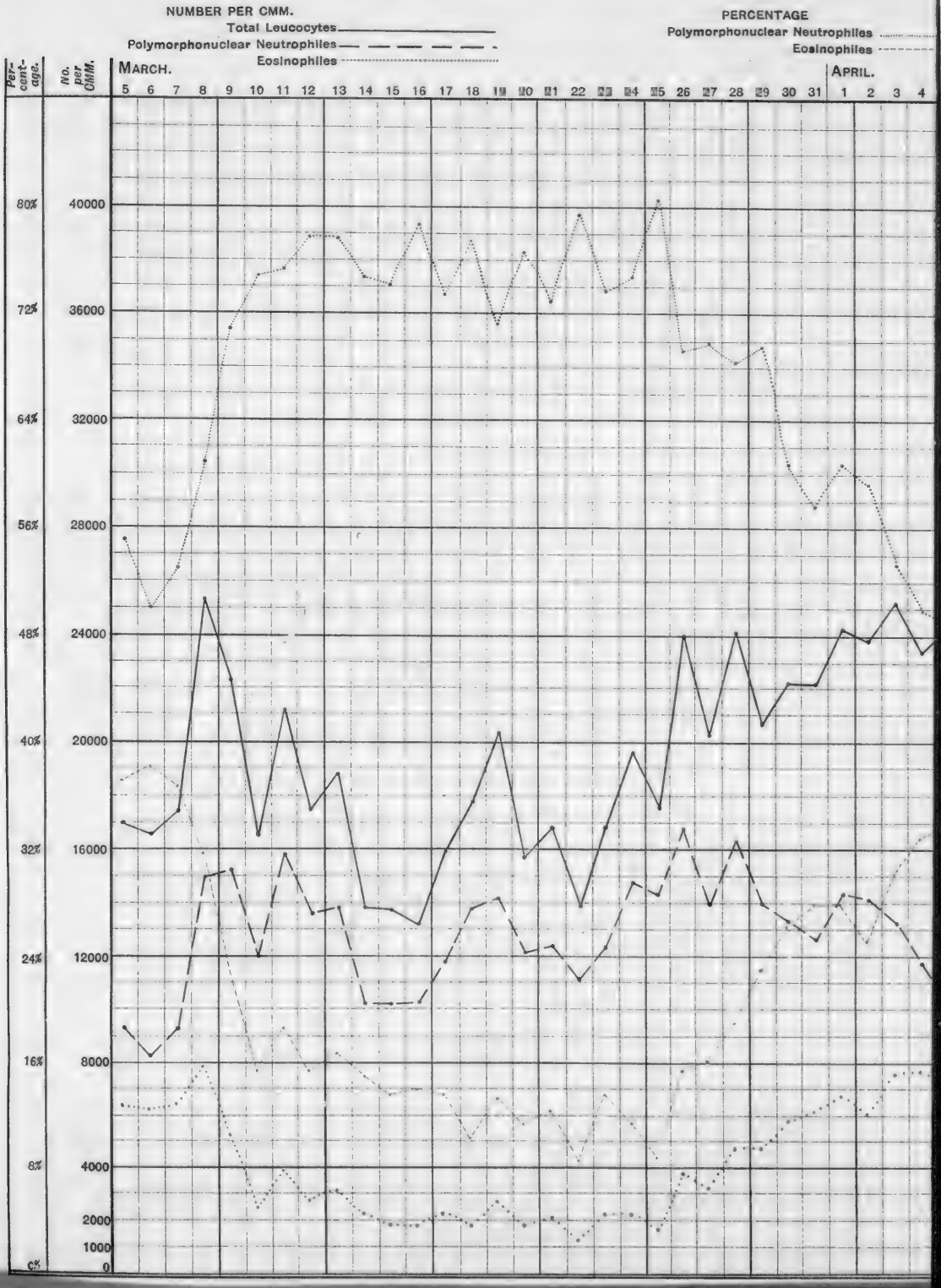
Showing Total Number of Leucocytes, of Polymorphonuclear Neutrophiles and of



LEUCOCYTIC CHART OF CASE I.

onuclear Neutrophiles and of Eosinophiles, and also the Percentage of Polym

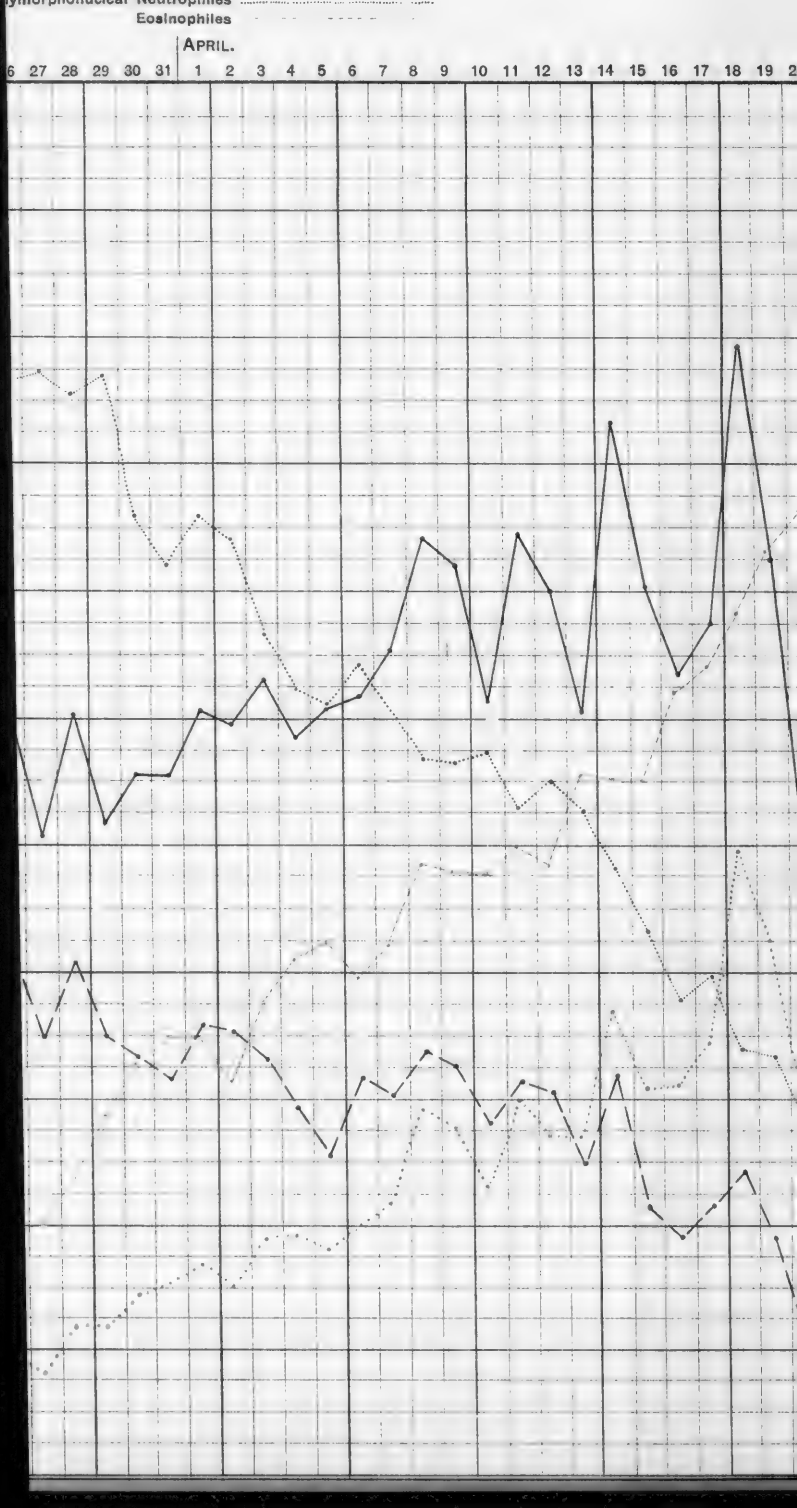
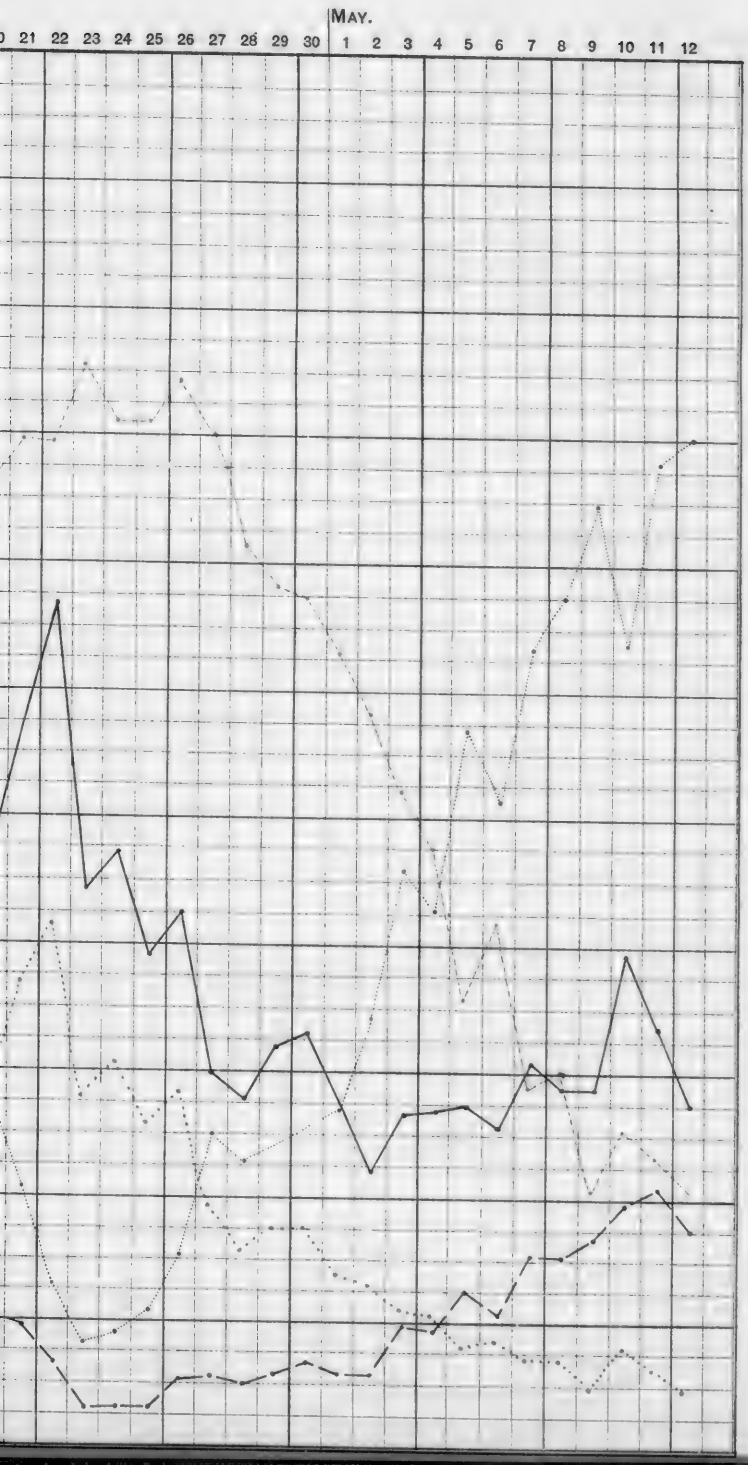


PLATE XXV.

orphonuclear Neutrophiles and of Eosinophiles.



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ON THE PRESENCE OF THE TYPHOID BACILLUS IN THE URINE.*

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The fact that the urine of individuals sick with typhoid fever may, at times, contain the specific organism of the disease has, during the past decade, occupied the attention of a considerable number of observers. The results obtained, and the conclusions drawn, are briefly as follows:

Bouchard,† in 65 cases of typhoid fever, found in the urine no bacteria and no albumin in 44. In 21 cases there were bacteria, and these were always associated with albumin. They were bacilli and were assumed to be typhoid organisms.

Hueppe ‡ succeeded in obtaining the typhoid bacillus from the urine in only one out of 18 cases.

Seitz § obtained positive results in 2 out of 7 cases. The urine of both positive cases showed albumin, and in one case also casts. The organisms, when found, were present in large numbers.

Konjajeff ‖ found typhoid bacilli in the urine of 3 out of 20 cases. Albumin was present in only one of these positive cases. The organisms appeared generally in the later stages of the disease (third week), and were always in large numbers. He thinks that the presence of the bacilli is always associated with the so-called typhoid lymphomata in the kidney.

Karlinski ¶ examined the urine in 38 cases in which the symptoms were those of typhoid and where the specific bacilli had been cultivated from the stools. He also examined bacteriologically the bladders at 6 typhoid autopsies. Out of these 44 cases the typhoid bacillus was isolated in 21, and was always associated with albuminuria. When found

* A partial report of investigations carried on under the provisions of the Dalton scholarship at the Massachusetts General Hospital.

† Bouchard, *Rev. de méd.*, i (1881), 671.

‡ Hueppe, *Fortsch. der Med.*, iv (1886), 448.

§ Seitz, Bakt. Studien zur Typhus-Aetiologie. München, 1886.

‖ Konjajeff, Ref. in *Centralbl. f. Bakt.*, vi (1889), 672.

¶ Karlinski, *Prag. med. Woch.*, xv (1890), 437 and 452.

the bacillus was always in pure culture. K. claimed to have obtained a positive result as early as the third day of the disease.

H. Neumann * out of 48 cases obtained positive results in 11. He found the bacilli always in large numbers, but thought he probably failed to detect them when their number was small. In his experience albumin was not necessarily an accompaniment of the bacilli. Moreover, albumin was often present without bacilli. Furthermore, Neumann pointed out the fact that typhoid bacilli can reproduce themselves rapidly, even in an acid urine, and that the bladder, with its constant supply of fresh urine, offers a peculiarly favorable place for the multiplication of the organisms. The prognosis was not necessarily worse in consequence of the presence of the bacilli in the urine. Neumann made the important statement, also, that the typhoid organisms may persist a considerable time after the cessation of fever, and that the urine of typhoid convalescents ought, therefore, to be subject to very careful supervision. He thought the bacilli were finally removed mechanically. In some cases cocci were found, and these were supposed to indicate a complication.

Geisler,† from a review of the literature, concluded that typhoid bacilli could be isolated from the urine in about 26 per cent of the cases.

Borges ‡ obtained positive results in 2 out of 10 cases. In both cases there was albumin in the urine. B. thought that the albumin and the bacilli disappeared together. In one-half of the cases there was evidence of pathological change in the kidneys, whereas in only one-fifth of the cases were there bacilli.

Baart de la Faille § obtained typhoid bacilli in the urine of 4 out of 27 cases. In 4 other cases he found colon bacilli.

G. Blumer || found pus in the urine in 10 out of 60 cases, and this pus was always associated with bacteria. The colon bacillus was present 7 times, 6 times alone and once together with the typhoid bacillus. The typhoid bacillus was found twice, once with the colon bacillus and once alone. The staphylococcus pyogenes albus appeared in one case, and in another an unrecognized coccus was observed. Blumer thought the bacteria reached the bladder perhaps through the recto-vesical wall.

* Neumann, *Berlin. klin. Woch.*, xxvii (1890), 121.

† Geisler, Ref. in *Centralbl. f. Bakt.*, xiii (1893), 767.

‡ Borges, Ueber die Durchlässigkeit der Typhusniere für den Bacillus typhi abdominalis. Inaug.-Diss., Würzburg, 1894.

§ Baart de la Faille, Inaug.-Diss., Utrecht, 1895. Ref. in *Centralbl. f. Bakt.*, xviii (1895), 337.

|| Blumer, *Johns Hopkins Hospital Reports*, v (1895), 327.

The pus in the urine he considered important only because of its possible association with the typhoid bacillus, and therefore recommended that the patients should be kept in the hospital till the pus had disappeared.

Wright * had the unusually good fortune to find typhoid bacilli in 6 out of 7 cases.

Besson † examined the urine of 33 typhoid patients and obtained typhoid bacilli in 6. In 12 urines containing only slight traces of albumin bacilli were found but once. In 12 cases with considerable amounts of albumin the bacilli were present in 5.

As we look over the writings of these observers we find that the following general facts were brought out by their work:

(1) The typhoid bacillus was demonstrated in the urine of typhoid patients in about 25 per cent of the cases.

(2) The bacilli, when demonstrated, were present nearly always in large numbers and, in the great majority of cases, were associated with albuminuria. On the other hand considerable amounts of albumin were often present in the urine without bacilli.

(3) The organisms appeared generally in the later stages of the disease, but, having appeared, they might persist for a considerable time into convalescence.

These conclusions, then, represent in the main the results obtained up to the year 1898.

Of course we must take into consideration the fact that in the early days of the literature relating to the typhoid bacillus the positive identification of this bacillus was much more uncertain than at present, and that confusion of the typhoid organism with other bacilli, especially those of the colon group, must have occasionally taken place. We must, therefore, view with some suspicion such results as those of Karlinski,‡ who obtained positive results in 50 per cent of his cases, and that, too, in very early stages of the disease. On the other hand the results of Wright§ were no less unusual (6 positive

* Wright, *Lancet*, 1895, ii, 196.
† Besson, *Rev. de méd.*, xvii (1897), 405.
‡ Karlinski, loc. cit.
§ Wright, loc. cit.

cases out of 7), although, in this instance, the more important of the modern differential tests were used.

The following details of my own investigations and results will be found to confirm substantially the conclusions stated above.

The methods employed in the work were briefly these: In the case of male patients (by far the larger number) the glans penis was first thoroughly cleansed with corrosive sublimate solution 1:1000. The urine was then passed in two portions into sterilized test tubes. Only the second portion was used for bacteriological purposes. The female patients were catheterized with all possible antiseptic precautions, and in these cases also only the second part of the urine was used.

The urine obtained in this manner was then plated, with as little delay as possible, upon plain agar. Two plates were made for each specimen, one representing a single loop of urine and the other 1 cc. (approximately). After being left for 18 to 24 hours in the incubator the plates were examined under a low power of the microscope (Zeiss AA or Leitz No. 3).

It might be thought that difficulties similar to those encountered in making cultures from the stools might be met with also in the case of the urine, and that, therefore, a differential medium, such as that of Elsner, Capaldi or Hiss, would be necessary in this work. But it has been found that in nearly all cases we have to do with but a single organism (rarely two or three), so that ordinary media answer our purpose perfectly well. The colon colonies are, naturally, the ones most easily mistaken for the typhoid, but a little experience makes it possible to tell them apart.

In cases of doubt I have made use of the following procedure with great satisfaction as a preliminary test: If we examine closely the rent in a typhoid colony, which we make in the process of inoculation with the platinum needle, we can see oftentimes, even with the low power, a marked seething-motion resembling very much that of a swarm of bees. This phenomenon is due, of course, simply to the setting free of the very motile bacilli, and becomes much more evident with a higher power of the microscope (Zeiss DD or Leitz No. 7). The amount of motility varies in different colonies, but it is very

rare that one is not enabled by this procedure to say whether the organism is or is not actively motile. In my experience with typhoid urine and fæces, motile bacteria of other varieties have not been sufficiently numerous to invalidate the procedure as a preliminary test.

The cultures of cocci isolated during the work were examined only as to their shape and arrangement. The typhoid and colon cultures were subjected to the following differential tests:

(1) Hanging drop for size, shape and motility.

(2) Growth upon gelatin slant and in stab cultures.

(3) Growth upon the tube medium of Hiss * for the presence or absence of general clouding and gas production.

(4) Growth in peptone solution. Presence or absence of indol.

(5) Growth upon potato. Presence or absence of visible growth.

(6) Growth in the two test solutions of Proskauer and Capaldi.

(7) Growth in litmus-milk.

(8) Reaction to typhoid serum. †

Coming now to a presentation of the results of the investigation we find that 172 specimens of urine, representing 38 cases of typhoid fever, were examined. Of these 172 specimens 44, representing 9 cases, showed the presence of typhoid bacilli. In 2 cases, one positive and the other negative as far as the presence of the typhoid organism was concerned, the urine was examined first at autopsy (Cases II and XXI).

The following tables give the results in detail. The numbers refer to the day of the disease as nearly as it could be calculated from the history. ‡

* *Journal of Experimental Medicine*, ii (1897), 677.

† In carrying out this test I have always made use of the dried serum, or "serum paper," described by me in the *Centralbl. für Bakt.*, xxi (1897), 445. The paper is now 14 months old and shows no appreciable decrease in its agglutinating power. It is certainly a most convenient form for preserving the test for laboratory use, especially in localities where typhoid cases are scarce, and where, therefore, fluid serum is not always to be had.

‡ I am indebted to the records of the Massachusetts General Hospital for my information as to the presence or absence of casts, and as to the clinical features of the cases.

TABLE I. CASES I TO IX.—POSITIVE CASES.

Number of case.	Sex.	Cultivations made from urine.	Typhoid bacilli found.	Albumin present.	Casts.	CLINICAL FEATURES. Rose spots.	Delirium.	Hemorrhage.	Bronchitis.	Cystitis.	Temp. normal.	Relapse.	Day of discharge.	REMARKS.
I	M	38.42.47.54. 59.63.67.70.72 78	38.42.47.54 59.63.67.70.72 78	38.42.54.59 63.67.70.78 always in faint traces.	8 = no casts. No record thereafter.	+	0	0	pneumonia 35	0	24	27 to 56	72	In relapse a pneumonia. Typhoid bacilli in sputum 44th, 47th, 49th days. Phlebitis 28th day.
II	M	34.	34.	5 = faint trace	15 = present.	+	+	++	+	0				On 33rd day symptoms of perforation. Operated upon. Died same night.
III	M	23.25.28.33 41	23.25.28.33 41	23.25.28 = faint traces. 41 = 0	3.19.29 = present.	+	0	0	0	0	27	0	44	During convalescence urine averaged 70 ozs. daily.
IV	F	33.38.43.53	33.38	33.38.43 = very faint traces 53 = 0	20.26.28 = present.	+	0	0	0	0	40	0	55	Catheterized upon 13th day.
V	M	20.30.36.40	36.40	30.36.40 = faint traces	10 = present.	0	++	+	+	very slight.				Died upon 43rd day.
VI	M	8.11.15.20.25 27.30.31.33 34.36.37.39 43.66	15.20.25.27 30.31.33.34 36	8.11.15.25 = a trace. 27.30.31.36.37 39.43.66 = faint trace.	8 = present. 30 = absent.	+	0	0	0	+	19	0	43	Catheterized on 8th and following days. Bladder irrigated with boric acid 2% on 30th and 33rd days. No effect on bacilli. On 36th day irrigation with corrosive sublimate 1 : 7000. No bacilli on 37th, 39th, 43rd, 66th days.
VII	M	12.15.17.24.28 33.40.49.59	24.33.40.49	12.15.17.24 = $\frac{1}{8}$% 28.33.40.49 59 = large trace.	6.12.18.35 = present.	0	0	0	0	0	48	0	59	Marked post-febrile polyuria.
VIII	M	12.24.31.32 37.45	32.37.45	12.37.45 = faint trace.	30.38 = No casts found.	0	0	0	0	0	29	0	48	Furunculosis. Cultivation showed staphylococcus aureus in pure culture.
IX	M	38.43.46.52.58 65.72	38.43.46.52 58.65.72	14.38 = trace. 43.46.52.58 65.72 = faint trace.	14.31.52 = present.	+	0	0	0	0	35	38 to 53	67	Catheterized on the 14th and following days. Epididymitis on the 45th day. Urine during last week in hospital averaged 80 ozs. daily.

TABLE II.—NEGATIVE CASES. CASES X TO XXXVIII.

Number of case.	Sex.	Cultivations made from urine.	Bacteria found.	Albumin present.	Casts.	CLINICAL FEATURES. Rose spots.	Delirium.	Hæmorrhage.	Bronchitis.	Cystitis.	Temp. normal.	Relapse.	Day of discharge.	REMARKS.
X	F	49.	0	49=large trace.	11.16=present.	0	+	0	+	0	27	42 to 53	60	
XI	M	24.32	0	24.32=0	0	0	0	0	0	0	30	0	43	
XII	M	18.23.26.31 40.54.65	0	18.23.26.31.40 54.65=0	0	+	0	0	0	0	28	46 to 58	79	
XIII	F	16.20.23 35.40.48	16.20.23.35=a diplococcus 40.48=colon bacillus.	8.16.23.46= faint trace. 25.35.40=a trace.	8.28.33 =present.	+	+	0	0	+	29	0	62	Catheterized for a considerable time.
XIV	M	14.26	0	14.26=0	0	0	0	0	0	0	25	0	38	
XV	M	26.38.45 54.57	0	26.38.45.54.57 =0	0	0	+	0	0	0	70	0	78	
XVI	M	15.29	0	15.29=0	0	+	0	0	0	0	24	24 to 31	53	Phlebitis on 39th day.
XVII	M	15.18.19.31 38	18.19.31=a streptococcus 38=colon bacillus.	15.18.19.31.38 =0	0	0	0	0	0	0	30	0	46	
XVIII	M	24.45	0	24.45=0	0	0	0	0	0	0	20	0	48	
XIX	M	20.25.33 37.40	20.25.33.37.40 =a diplococcus.	20.25.33.37.40 =0	0	+	+	+	0	0	28	0	52	Pulse reached 147 at one time.
XX	M	24.31.39	0	8.24.31=0 39=ft. trace.	8=present.	0	+	0	+	0	28	0	40	
XXI	M	38	0	25=very faint trace.	25=present.	0	+	0	0	0			Died 38	Culture made from bladder at autopsy. A very severe case with high fever and pulse rate.
XXII	M	24.32.38	24.32.38= colon bacillus	24=faint trace.	0	+	0	0	0	+	26	0	44	Catheterized on 18th day.
XXIII	M	9.16.24.33	0	9.16.24.33=0	0	+	0	0	0	0	20	0	36	

TABLE II.—*Continued.*

Number of case.	Sex.	Cultivations made from urine.	Bacteria found.	Albumin present.	Casts.	CLINICAL FEATURES. Rose spots.	Delirium.	Hemorrhage.	Bronchitis.	Cystitis.	Temp. normal.	Relapse.	Day of discharge.	REMARKS.
XXIV	M	20.27		20.27=0										
			0		0	0	0	0	0	0	20	0	31	
XXV	M	10.21		10.21=0										
			0		0	0	0	0	0	0	21	0	34	
XXVI	M	9.14.22.29	9.14.22.29.36=	9.14.22.29.36	15=present.									Bladder operated on a year before entrance. Came in with cystitis.
		36	colon bacillus.	=faint trace.		0	0	0	0	+	30	0	46	
XXVII	M	42.45		33.42.45=0	33=present.									Probably came in during a relapse.
			0			0	0	0	0	0	35	+	46	
XXVIII	M	18.23.27	18.23.27.34.41=	15.18.23.27	15=present.									
		34.41	a diplococcus.	=faint trace.		+	0	0	0	very sl.	38	0	60	
			41=0.	34.41=0										
XXIX	M	18.22.25		18.22.25.27=0	0	+	0	0	0	0	28	0	47	
		27.33	0	33=faint trace.										
XXX	M	23.25.32		23.25=ft. trace.	21.28	0	+	0	0	0	25	0	45	
			0	32=0	present.									
XXXI	M	21.25.31.33		21.25.31.33.35.39	0									
		35.39.42.54	0	42.54=0		0	+	0	0	0	45	0	65	
XXXII	F	24.28.30	44.51.56=	21=$\frac{1}{4}$% 24.28.30	21=present.									Catheterized.
		35.44.51	colon bacilli.	38.44.51.56		0	+	0	0	sl.	56	0	72	
		56		=large trace.										
XXXIII	M	13.15.17.27		13.15.17.27=0										
			0		0	0	0	0	0	0	24	0	42	
XXXIV	M	25.36.51		25=faint trace.	20=present.									
		53	0	36.51=0		+	sl.	0	0	0	44	0	56	
XXXV	F	24	24=	9=$\frac{1}{6}$%.	9=present.									
			colon bacillus											
			25=			+	+	+	+	0			25	
			colon bacillus (autopsy).										Dead.	
XXXVI	M	9.15.20.34		9.15.20.34.49	49=present.									
		49	0	=faint trace.		+	0	0	0	0	37	0	52	
XXXVII	M	15.19.24		15.19=ft. trace.	23=present.									
		37.44	0	24.37.44=0		0	0	0	0	0	36	0	50	
XXXVIII	M	33.38.43		33.38=trace.	30=present.									
		51.57	0	43=faint trace.		0	+	0	+	0	43	0	71	
				51.57=0										

From an examination of the table of positive cases (I to IX) we see that, in 5 out of the 9, the bacilli were found on the first day of cultivation. How much earlier they may have been present it is, of course, impossible to say. The other 4 positive cases, although examined from a comparatively early period of the disease, showed no bacilli until the 15th, 24th, 32nd and 36th days respectively. The invasion of the urine by the typhoid organism seems to be, therefore, a late rather than an early occurrence. For this reason the examination of the urine bacteriologically for the purpose of diagnosis would seem to have but little value as compared with the specific serum test. All the cases under consideration showed at some time during the disease the so-called Widal serum reaction.

A more important point, and one to be very strongly emphasized, is the length of time that the bacilli may persist in the urine after its infection. Of the 9 positive cases 7 survived the disease and were discharged as well. In 5 of the 7 cases, however, the urine still contained, upon the day of discharge from the hospital, typhoid bacilli in undiminished numbers. This fact would be, perhaps, of less importance if each loop of urine harbored one or two bacilli, but when it is considered that in most cases a single loop contained many hundreds, or even thousands, of organisms, then the matter assumes an entirely different aspect, especially when viewed in its relation to disinfection and the public health. Modern sanitary regulations forbid that diphtheria patients in whose throats Klebs-Löffler bacilli persist shall be suffered to go abroad. Is it not just as important that there shall be a similar supervision of convalescent typhoids, who may be passing with every micturition pure cultures of the typhoid bacillus?

These patients were given careful instruction as to the disinfection of their urine and were urged to send weekly specimens for further examination; but, as so often happens, they have carried out these instructions very unsatisfactorily. In 2 cases the urine 10 days subsequent to discharge still showed bacilli. In another case after a similar interval the organisms had disappeared. Two cases have yet to be heard from. It is, therefore, impossible to say just how long

the bacilli may persist in these typhoid urines, but it is evident that they remain long enough to make the infected individuals a source of danger to those in their environment.

Neumann thought that the bacilli were finally removed mechanically. Assuming, as seems probable, that the large number of bacilli is due, not to excretion as such by the kidney, but to rapid multiplication in the bladder, it would seem that the post-febrile polyuria, with frequent and copious micturition, would, in the end, tend to eliminate the organisms entirely. This theory would explain, perhaps, the disappearance of the bacilli in Case VII. On the other hand Cases III and IX averaged during convalescence 70 and 80 ounces of urine daily, without, however, any apparent diminution in the number of bacilli. Nature does not, therefore, provide properly for this condition. Can we, by any method of treatment, render these cases innocuous?

My experience upon this point is confined to observations upon a single case (VI), in which the bladder was washed out with antiseptic solutions. At first boric acid was tried in the strength of 2 per cent, but, although the solution was retained for 2 or 3 hours, no effect upon the bacilli could be observed. Finally corrosive sublimate 1:7000 was injected, and although the fluid was passed out again almost immediately, no bacilli could be discovered during the 30 days following. It would seem, therefore, that in antiseptic irrigation of the bladder we have a possible means of freeing these cases of their dangerous character. Whether a like result can be obtained by the internal administration of salol or other similar drugs must be determined by further observation.

From a further examination of Table I it will be seen that 3 of the positive cases had been catheterized early in the disease. This offers the possibility, of course, of a typhoidal infection from without. The fact, however, that in these cases the bacilli were always found in pure culture and unaccompanied by colon bacilli or other bacteria would, as it seems to me, speak almost certainly for their internal origin.

Case IX is especially interesting as having developed during con-

valescence an epididymitis. A venereal origin, gonorrhœal or otherwise, was entirely improbable from the history, and the most plausible theory was that the affection of the epididymis was typhoidal in character, having its origin in the infected urine. The inflammation subsided without surgical interference, so that conclusive bacteriological investigation was impossible.

To proceed with the analysis of the tables, we find that the presence of bacilli in the urine was practically always accompanied by albuminuria, but that in nearly all the cases the amount of albumin was small, in only one case reaching $\frac{1}{8}$ per cent.

Of the 29 cases in which the urine was negative, so far as typhoid bacilli were concerned, 15 had albumin in the urine, and that, too, in amounts larger, on the average, than those containing the typhoid organisms (XXXII, $\frac{1}{4}$ per cent; XXXV, $\frac{1}{6}$ per cent).

As to the presence or absence of casts, they were found in all albuminous urines except those of Cases I and VIII. The record of Case I is decidedly incomplete, however, and it is more than probable that casts were present at some time during the disease.

No parallel can be drawn, therefore, between the presence of albumin and casts and the presence of typhoid bacilli in any urine. On the other hand we can say that a non-albuminous urine very probably contains no typhoid bacilli. Case III was the only exception in this series, showing no albumin upon the 41st day in the presence of many typhoid organisms. Furthermore, Case VII demonstrates the fact that albumin can persist after the disappearance of the bacilli.

Of the 29 cases in which no typhoid bacilli were found, 8 showed bacteria of other kinds. Of these 8 cases, 4 (XVII, XIX, XXVIII, XXXV) may be assumed to have derived their organisms from within the body. In the other 4 cases (XIII, XXII, XXVI, XXXII) the bacteria may have been of external origin (catheterization, operation). In these 8 cases a diplococcus (not further identified) was found in 2 (XIX, XXVIII); colon bacilli alone in 4 (XXII, XXVI, XXXII, XXXV); a diplococcus followed by colon bacillus in one (XIII); a streptococcus followed by colon bacillus in one (XVII). The colon

bacilli presented no special points of interest except that isolated from Case XXVI. This organism was moderately motile, and grew upon litmus-milk, potato, gelatin and sugar-agar in a manner exactly similar to that of the typhoid bacillus. It might, therefore, according to criteria in vogue previous to 1890, have been called the typhoid organism. It was found, however, that upon peptone solution the bacillus produced immense amounts of indol; that it failed to react to typhoid serum; that it grew with acid reaction upon solution No. 1 of Proskauer and Capaldi, and that it failed to produce general cloudiness upon the tube medium of Hiss. This organism illustrates, then, forcibly the point referred to above, namely, that the results of investigations made in the eighties must be taken with a certain amount of reserve.

Cystitis was present in 7 cases and, with one exception (V), was always associated with bacteria and with a history of catheterization or operation. On the other hand the urine of 11 cases contained many bacteria with no evidence of cystitis.

In a general way the cases which showed typhoid bacilli in the urine were of a more severe type, as evidenced by the clinical course and mortality. Especially to be noted is the tendency to relapse and complication (I and IX).

The finding of typhoid bacilli in the sputum (I) is of interest in its possible etiological relation to the pneumonia which was present in this case.

Finally, the presence of typhoid organisms in the urine casts doubt, from a strictly scientific point of view, upon the work which has been done upon typhoid stools. Certainly, in my own investigations of the fæces in typhoid fever,* I never took into consideration the possible origin of the bacilli from the urine, and no effort was made to exclude the urine from the specimens examined. Nor have other observers, so far as I am aware, mentioned this possibility of error. From the point of view of diagnosis, of course, it makes no difference whether the bacilli come from the fæces or the urine.

* *Boston Med. and Surg. Journal*, Oct. 28, 1897.

CONCLUSIONS.

(1) Typhoid bacilli were demonstrated in the urines of 9 out of 38 typhoid patients (about 25 per cent). 172 specimens were examined with 44 positive results.

(2) The bacilli, when demonstrated, were always present in large numbers and in practically pure culture.

(3) The bacilli appeared first in the later stages of the disease and persisted in the great majority of cases far into convalescence.

The urines of typhoid patients should, therefore, not only be rigidly disinfected during the disease, but they should also be carefully supervised during convalescence.

(4) The typhoid bacilli were practically always associated with albuminuria and the presence of renal casts. On the other hand urines containing considerable amounts of albumin and casts in large numbers often showed no typhoid bacilli.

(5) Irrigation of the bladder with antiseptic solutions offers a possible means for removing permanently the bacilli from the urine.

In conclusion I wish to express my indebtedness to Dr. J. H. Wright, Director of the Pathological Laboratory, and to the physicians of the visiting and house staff at the Massachusetts General Hospital, for their kind co-operation in the work.

THE BRANCHED FORM OF THE BACILLUS TUBERCULOSIS IN SPUTUM.

By CHARLES F. CRAIG, M. D., Danbury, Conn.

PLATE XXVIII.

The first* to draw especial attention to branching and budding forms of the tubercle bacillus were Nocard and Roux (1) in 1887. In the following year Metchnikoff (2) described and figured long, thread-like, branching forms with bulbous ends, observed in old glycerine-agar cultures cultivated at 43.6°-44° C. It is probable that these were cultures from avian tuberculosis. Metchnikoff suggested the name Sclerothrix kochii for the tubercle bacillus. In 1889 Klein (3) described and photographed filamentous, club-shaped and branching tubercle bacilli in old cultures, and S. G. Dixon (4) reported making in Klein's laboratory observation of these forms. In 1892 A. Maffucci (5) described and depicted similar forms of the bacillus of fowl tuberculosis, which he considered to be thereby distinguished from the bacillus of mammalian tuberculosis. Especial attention was directed to pleomorphism and variability of the tubercle bacillus by the monograph of Fischel (6) published in 1893, in which he described in detail thread-like, branching and club-shaped forms both of mammalian and of avian tubercle bacilli, and emphasized the analogies between this organism and the ray-fungus of actinomycosis. Among the most valuable contributions to this subject are those of Coppen Jones (7), dating from 1893. In glycerine-agar cultures three to four months old he finds constantly filamentous, branching forms of the tubercle bacillus, and directs especial attention to the

* Cornil and Babes noted and pictured ramification and swelling of the tubercle bacillus in the first and second editions of their "Bactéries" in 1883 and 1886. According to Coppen Jones, A. Petrone in 1884 observed in the meningeal exudate several forms of the tubercle bacillus described in 1895 by the former, and expressed the opinion that it belongs to a group situated between the "micromycetes and the schizomycetes."

regular arrangement of the rods and threads in cultures whereby marked resemblance to actinomyces colonies is produced, a resemblance still further increased by the presence of bulbous swellings on the free projecting ends of the tubercle bacilli. He concludes that the tubercle bacillus is to be placed in the same class of organisms as the ray-fungus and cannot properly be regarded as belonging to the schizomycetes. Since the publications of Fischel and of Coppen Jones a number of confirmatory observations, although with much difference of opinion as to their interpretation, have been published, among which may be especially mentioned the investigations of Bruns (8), Babes (9), McWeeney (10), Semmer (11), Marpmann (12), Johan-Olsen (17), Babes and Levaditi (13), and Friedrich (14). Babes and Levaditi, and Friedrich have succeeded in demonstrating in tubercles produced by intracranial and intravascular injection of tubercle bacilli typical ray-like colonies of the bacilli very closely resembling those of actinomyces.

Branching, filamentous and bulbous forms of tubercle bacilli have been observed most commonly in old cultures, although they have been seen by Fischel, Coppen Jones and others in material from tuberculous cavities and in sputum. Recent observations, as already mentioned, show that under certain special conditions they may occur also in the tissues. In view of the interest of the subject and the relatively small number of reports of finding branched forms in the sputum, it has seemed to me desirable to record my observation of such forms in this situation.

The sputum was from a young woman suffering from rapidly advancing pulmonary phthisis. The expectoration was abundant and of offensive odor, containing, besides numerous bacilli, large quantities of elastic tissue.

The stained specimens were prepared as follows: A minute portion of the sputum was spread upon a cover-glass and another glass drawn gently over the first; the two specimens thus obtained were allowed to dry, passed rapidly three times through the alcohol flame, and then stained several hours, generally twenty-four, with the following solution:

Fuchsin	1 part.
Alcohol	10 parts.

Dissolve the fuchsin in the alcohol and add 100 parts of a 5 per cent aqueous solution of carbolic acid.

When rapid staining was desired a portion of the staining solution was poured into a watch-glass, the specimens to be stained being placed face downward upon it; the watch-glass was then gently heated until steam was given off freely, then placed aside and the specimens allowed to stain for about ten minutes longer. After staining, the preparations were washed in distilled water and decolorized with a 20 per cent nitric acid solution in alcohol; they were then washed in alcohol, dried and mounted in balsam or examined directly. Specimens stained by either of these methods gave excellent results, the tubercle bacilli being colored a very dark red, all else being decolorized.

In almost every preparation made from this sputum there were observed several bacilli showing true branching, and also those showing what I believe to be intermediate stages between the simple and the branched rod. While the typical straight or slightly curved bacilli are, of course, much the more numerous, the branched and intermediate forms are present in almost every microscopic field and are easily demonstrated. The appearances presented by the various branched and intermediate forms are very numerous and it would be impossible to depict them all, but the drawings reproduced in Plate XXVIII show the more striking variations.

In very many of the preparations there may be seen bacilli like those shown in Plate XXVIII, Fig. 1, and which are evidently false branching forms. On casual examination the resemblance which these bacilli (for they are multiple) bear to the true branching form is very striking, and it needs very careful focussing and good illumination, in many instances, to distinguish the difference. This appearance is due not to a single bacillus sending off branches, but to two or more bacilli lying very closely together and in such a position as to give the impression of true branching. Careful examination will, however, in almost every instance disclose a slight, unstained interval at the point where the suspected branching takes place. Such a minute examination should be made of every bacillus which appears to branch. Where no unstained interval can be detected by reason of one end of the bacillus overlapping another, careful focussing will show a difference in the level of the bacilli where they overlap, and also a deeper staining at that point, whereas in the true branching form no such difference in level or staining can be detected. Another distinction exists in the fact that in the false-branching type the bacilli forming the supposed branches are of the same length as the parent germ, whereas in the branching form the branches

may be much longer or shorter than are the bacilli from which they spring.

Of the true branching bacilli several forms may be observed, which are doubtless simply stages in the growth of the organism. In certain fields there are seen bacilli which show at one or more points small knob-like projections, which cause a marked bulging in the outline of the organism. This form is illustrated in Plate XXVIII, Fig. 2, and evidently indicates the very first stage of branching, *i. e.* budding. These budding forms were not numerous in the preparations examined, but occurred in small numbers in the majority of the microscopic fields. They need sharp focussing and a good light to be distinguished, as in some of them the bulging is very slight and only such careful examination will demonstrate them. The bulging or knob is generally situated laterally, but may be central. The bud, as it may be called, stains like the rest of the bacillus, and sometimes a very small, oval, unstained speck may be seen within it which resembles the unstained portions in the bacillus. Generally, however, no such appearance is present, the bud staining uniformly throughout. Is this unstained oval speck a spore? I believe not, any more than are the similar unstained spots which occur in tubercle bacilli in general and which are not regarded by the best observers as spores.

The budding form just described is of interest, as it is doubtless the first stage of branching, and I believe it is present more often than is generally known. I have examined a large number of specimens of phthisical sputa since I first observed this form and have found it present in about 15 per cent of the cases, although in none was the branched form present, save in the one case described in this paper. This result may appear contradictory if we assume that the budding is the first stage of the branching form, but it is probable that some unknown factor has hindered the further development of the process of branching.

The next stage in the development of branching is shown in Plate XXVIII, Fig. 3. All the preparations made from the sputa in this case show numerous examples of this form. Here the little knob-like outgrowths, or buds, have developed into short branches, which vary in number in different bacilli, some giving off only one branch, while others give off two or more. The branches, at this early stage, are generally shorter than the parent bacillus and stain, almost without exception, uniformly throughout; the parent bacillus, however, generally shows intervals which take the stain very faintly or not at all. The bacillus itself, regardless of the branches, is much longer than usual, and somewhat more curved; they stain more deeply than the normal bacilli and resist a stronger decolorizing solution.

In nearly all the preparations of this sputum, the next stage, the fully developed branching form, can be seen (Plate XXVIII, Fig. 4). We have here simply a more advanced stage of the process, the short branches having developed into those which are as long as, or longer than, the parent bacillus. Of these fully developed branched forms there are present many varying examples; sometimes only one long branch is given off, sometimes two or more, sometimes one or more long branches and several minute buds; occasionally bacilli are seen where the primary branch has given off one or more secondary branches.

At *a* in Fig. 4 may be seen a bacillus which presents a well developed branch and three bud formations; this clearly indicates that the "bud" is the preliminary stage of the branch. It will be noticed that whereas the "buds" have taken the stain very deeply, the fully developed branch is stained much more faintly.

At *b*, in the same figure, is illustrated a bacillus presenting two short branches and one bud formation. Some of the bacilli are very fantastic in appearance, being much curved and giving off one or more branches, also curved (*d*, Fig. 4). Nearly all the bacilli present well marked stained and unstained areas, but it is noticeable that the branches present fewer of these, although some show unstained areas, irregular in outline. The bacilli are much longer than usual and many of the preparations show single filaments which are very long. In one preparation a peculiar appearance is noted (*g*, Fig. 4), which seems to represent the union of two branched bacilli by an intervening branch. This probably is an exaggeration of sub-branching. Many of the bacilli show sub-branching, the original branch giving off one or more smaller branches.

It is possible, then, to trace in these preparations from sputa the process of branching as it occurs in the bacillus tuberculosis. The bacillus destined to present this change becomes longer than usual and presents one or more knob-like outgrowths somewhere along its course; these outgrowths gradually lengthen and at last become true branches, which may, in turn, give off other branches.

We are not informed as to the special conditions which determine the development of branching forms of the tubercle bacillus. They have been most frequently observed under unfavorable conditions of environment, as in old cultures. In the present case it may be that similar factors are involved, as the rapid breaking down of tissue which characterized the process in the lungs may have exhausted the pabulum for the numerous bacilli present. Still the recent observations of Babes and Levaditi and of Friedrich show that similar forms may occur in fresh

miliary tubercles. Various theories have been advanced as to the significance of the branching, filamentous and club-shaped forms of the tubercle bacillus.

Some have considered these to be simply degenerative or pathological forms of the bacillus and without significance as to the classification of the organism. This is the view advocated by A. Fischer (15) in his "Vorlesungen über Bakterien," who places these forms of the tubercle bacillus, as well as similar ones of the diphtheria bacillus, in the group of involution forms. There are several objections to this view, of which it will suffice to mention that the forms in question are not limited in their occurrence to old cultures and unfavorable conditions of environment.

The opinion more generally advocated is that the occurrence of these forms necessitates some change of formerly accepted views as to the classification of the micro-organisms concerned. Thus Klein suggests that the branching is a reversion to an ancestral type which presented mycelial growth.

Fischel pointed out the analogies between actinomyces and the tubercle bacillus, and Coppen Jones holds that both belong to the same group of organisms, but he leaves undecided whether this is among the hyphomycetes or some other class. He suggests that there may be some as yet unknown saprophytic mode of existence of this bacillus. He suggests the name Tuberculomyces (hominis, avium, etc.) for the bacillus.

Bruns regards the branching shapes as involution forms when observed under parasitic conditions. He says "they pertain to the saprophytic growth-form of a higher organism which, as a parasite in the animal body, appears in the form of rods." Marpmann, accepting the interpretation of Bruns, employs the designation Mycobacterium tuberculosis, a name adopted by Lehmann and Neumann in their "Grundriss der Bakteriologie."

The sharper differentiation within recent years of the streptothriceæ, cladothriceæ and allied organisms has led several writers to express the opinion that the tubercle bacillus and the diphtheria bacillus are very closely allied to the streptothriceæ, and some hold that they should be transferred to this group. Kruse (16), in the last edition of Flügge's "Mikroorganismen," discusses this question and, although he does not venture to place the bacteria in question under the streptothriceæ, he considers them to be very closely related to actinomyces and other organisms belonging to this group. Johan-Olsen (17) places the tubercle bacillus under the streptothriceæ and accepts the name Tuberculomyces. The streptothrix group is characterized especially by branch-

ing, filamentous forms of organisms, and is placed between the bacteria proper and the hyphomycetes, being more closely akin to the former than to the latter.

It is evident that further investigations are needed in order to enable us to come to any definite conclusion as to the significance of the branched forms considered in this paper, and for the present it would seem to be premature to adopt any radical change in the accepted classification of the tubercle bacillus and the diphtheria bacillus.

DESCRIPTION OF PLATE XXVIII.

Fig. 1. False branching form of the Bacillus tuberculosis in sputum. Careful examination shows an appreciable interval between the organisms. × 1000.

Fig. 2. Budding form of the B. tuberculosis in sputum. The little elevations or "buds" are probably the earliest evidence of branching. × 1200.

Fig. 3. Branching forms seen in sputum. × 1200.

Fig. 4. Various branching forms in sputum. × 2000.

a. An organism presenting one branch and three bud formations.

b. Organism showing two branches and one bud formation.

c. Organism showing the stained and unstained areas in its protoplasm.

d. Organism presenting two branchings, one of which stained uniformly, the other presenting unstained areas.

e. A branched form of the bacillus.

f. A bacillus showing one branch and two sub-branches, with another branchless bacillus lying beside it.

g. A peculiar formation probably due to the junction of two branched bacilli.

h. V-shaped form of the organism, showing two bud formations.

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FIG. 1.

FIG. 2.

FIG. 3.

a

b

c

d

e

f

g

h

FIG. 4.

The Friedenwald Company, Engravers and Printers.

THE EFFECTS UPON THE BLOOD OF THE TUFNELL METHOD AND THE CALCIUM SALTS IN THE TREATMENT OF AORTIC ANEURISM.

By ALONZO ENGLEBERT TAYLOR, M. D., Associate in Clinical Medicine.
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The following studies constitute a partial report of investigations which have been undertaken with the hope of determining the exact effects upon the blood of the current treatments of aortic aneurism. Drs. Pepper and Tyson placed at my disposal three such cases, one of whom died of rupture before the studies were completed, so that the report comprises but two cases.

The capillary and venous blood of the patients was first studied before any treatment was instituted, the natural volume of their urine noted, and the renal elimination of calcium accurately determined. The patients were then placed under treatment and the same determinations again carried out. In each case two sets of determinations were carried through both before and during treatment, with the exception of the estimations of urinary calcium and of the coagulation time of the capillary blood, which were performed every three days while the investigation was in progress.

The treatment consisted of rest, a dry diet, restriction of liquids, regular venesections (every 18 days), and the internal administration of potassium iodide grm. i and calcium chloride grm. ii daily. The use of a dry scant diet, restriction of liquids, and venesections are old therapeutic ideas, employed especially by Valsalva and Albertini; it is, however, due to the advocacy of Tufnell that they have held sway in modern practice. These measures have been held to reduce the volume of the circulating blood and to increase its coagulability. It cannot be believed today that therapeutic venesection more than momentarily diminishes the volume of the circulating blood, so

quickly is the amount abstracted replaced by fluids from the tissues; in fact it is not improbable that the lymph current may more than replace the volume withdrawn by the bleeding. For the rest there is more definite evidence. Withdrawal of all drink or profound catharsis soon leads to an oligohydræmia. I have, however, been able to find no studies tending to show that the degree of limitation of fluids practised in cases of aneurism has actually caused such an inspissation of the blood. Tufnell's own regimen did not include over 375 cc. of liquid in the food and drink, which under ordinary circumstances would not furnish over 300 cc. of urine. Such a regimen is extremely distressing. In the cases here reported it was not possible to reduce the patient's liquids below the urinary equivalent of 400 to 500 cc. per diem, and even that was very irksome. Whether or not a reduction of the water in the circulating blood renders it more coagulable is entirely an open question; I know of no experimental work tending to show that simple oligohydræmia *per se* is accompanied by an increase in the coagulable properties of the blood. Potassium iodide was administered for the relief of pain, without expectations of any action upon the blood. The employment of calcium salts to heighten the coagulating properties of the blood, so warmly advocated by Wright, is based upon the now generally admitted fact that calcium bears in some way an integral relation to the molecule of fibrin.

The methods employed by me were as follows: The calcium in the urine was estimated by weighing as calcium oxide after precipitation as calcium oxalate, according to established procedures. The specific gravity of the blood was determined by accurate weighing in a pienometer of a capacity of about 12 grm. of water. The calcium in the blood was estimated by evaporating about 75 cc. of blood to dryness; the residue was then incinerated, the ash dissolved in hydrochloric acid, diluted and neutralized; the phosphates were then removed by the addition under heat of ferric chloride in the presence of acetic acid and sodium acetate; the calcium in the filtrate was then precipitated as the oxalate, reduced to the oxide by heat and weighed.

The volume of plasma and corpuscles was determined by the Bleibtreu method. In this method two or more portions of blood are mixed in

different known proportions with an isotonic * saline solution, the cells allowed to sedimentate, and the nitrogen of the clear plasma in the different mixtures estimated by the Kjeldahl method or the specific gravity determined. Since the respective quantities of blood and saline solution are known, and since the dilution occurs (theoretically) in the plasma alone, the volume of the plasma, and from it that of the corpuscles, is calculated according to the following formula:

$$N^2 \frac{s^2}{b^2} - N^1 \frac{s^1}{b^1} = (N^1 - N^2)\, x.$$

N^1 = nitrogen of mixt. 1. N^2 = nitrogen of mixt. 2. b^1 and b^2 = volumes of blood in the dilutions. s^1 and s^2 = volumes of solution in the dilutions. Technically this procedure is accurate. In principle, however, it is not accurate unless the diluting fluid have the same osmotic density as the plasma of the particular blood under consideration, otherwise there will be an exchange between the cells and the plasma.† Whether this exchange is always in one direction or not has not been determined; so far as clearly demonstrated, the exchange seems to be from the cells into the plasma. The extent of this exchange is certainly not marked; the amount of nitrogen involved is so small in comparison to the quantity of nitrogen in the native plasma that the method with its acknowledged error remains much more accurate than those of sedimentation and centrifugation. The error was minimized, I believe, by diluting the blood with small quantities of saline solution to which potassium oxalate was added to prevent coagulation, and then quickly removing the cells by centrifugation so that only a few minutes elapsed from the time the blood was drawn from the vein until the plasma was transferred to the Kjeldahl digestion flasks. The diluting fluid employed was a 4 per cent oxalate solution in a 0.9 per cent NaCl solution. The first mixture was of large quantity, since it also served for the estimation of the fibrin, usually 4 cc. of the solution to about 96 cc. of blood; the second mixture was usually in the proportion of 2 parts of the solution to 25 parts of blood. I have often

* The word "isotonic" is here employed in its exact sense, related to osmotic density.

† If the osmotic density of the plasma of the particular blood be determined, preferably by the estimation of the reduction of the freezing point, and the saline diluting fluid made to correspond thereto, the result will be accurate. In the use of such solutions whose osmotic density corresponds to that of human plasma on an average (as 0.91 per cent. sodium chloride or 1.42 per cent. sodium sulphate) there will in the individual cases probably always be some exchange between the cells and the diluted plasma.

made smeared preparations of the corpuscles at the moment when the diluted plasma was drawn off for nitrogen analysis and found the corpuscles as a rule of normal appearance and staining reactions. At the end of an hour, however, the appearances of the red cells began to be altered, and these alterations progressed as time elapsed, and corresponding thereto later analyses of the plasma have always given higher quantities of nitrogen. I likewise made comparative determinations with the hæmatokrit, both with diluted and undiluted blood, and the results convinced me that the Bleibtreu method amply repays in accuracy the labor it involves.

From the plasma-nitrogen the amount and percentage of proteids in the plasma may be calculated, and in connection with the volume of plasma the amount of albumin in the plasma of 100 cc. of blood may be easily determined. These factors are of value, since any alteration in the quantity of water in the plasma would at once affect the quantity of albumin. There is in normal plasma a small quantity of non-proteid nitrogenous substances. In my cases the plasma has on an average contained azotized substances not precipitable by tannic acid corresponding to 0.060 N in 100 cc. of plasma, and that amount has been regularly deducted from the plasma-nitrogen before converting it into terms of albumin.

The fibrin was estimated by the recent method of Kossler and Pfeiffer.* A known quantity of venous blood, about 100 cc., is rendered non-coagulable by the addition of from 4 to 6 cc. of a 4 per cent solution of potassium oxalate in 0.9 per cent NaCl solution. The cells are immediately removed by centrifugation and the plasma-nitrogen determined by the Kjeldahl method (this also serves as mixture 1 of the Bleibtreu method). To 20 cc. of the oxalate plasma are then added 3 cc. of a 2 per cent calcium chloride solution. Spontaneous coagulation occurs rapidly; following the complete separation of the fibrin and the serum, the nitrogen of the serum is determined by the Kjeldahl method. Since the volume of plasma, the oxalate dilution of the blood and the calcium dilution of the oxalate plasma are known, the difference between the plasma-nitrogen and the serum-nitrogen with consideration of the aforementioned factors represents the nitrogen of the fibrin in the native volume of blood, the formula being as follows:

$$Nf = \frac{v}{v - v^1}\left(Np - Ns\,\frac{p + c}{p}\right).$$

Nf = fibrin nitrogen in 100 cc. blood. v = volume of oxalate plasma

* Kossler and Pfeiffer, *Centralbl. f. innere Med.*, xvii (1896), 1.

(Bleibtreu method). v^1 = amount of oxalate solution in 100 cc. of oxalate blood. Np = nitrogen in 100 cc. of oxalate plasma. Ns = nitrogen in 100 cc. of the diluted serum. p = quantity of oxalate plasma in the artificial coagulation. c = quantity of calcium solution in the same. Since we do not know the formula for the molecule of fibrin, the findings must be expressed in terms of nitrogen. This method is very accurate, though technically quite difficult.

The time of coagulation was determined by Wright's method. A dozen glass tubes of $\frac{1}{4}$ mm. dimensions are supported in glass or rubber cases and suspended in water kept at body temperature. An assistant noting the time, the numbered tubes are successively employed as follows: from a deep puncture in the finger the blood is drawn one-third the length of the tube, which is then removed from the finger and the column of blood drawn up so that it occupies the middle third of the tube, which is then replaced in its warm case. After a lapse of about two minutes (a preliminary orientation is always advisable) the tubes are successively blown out and the coagulation point noted, the time of which can then be calculated from the records. The range of error in this method is fully forty seconds, that is, it may be 20 seconds to either side of the given result. From studies on normal male adults I have found the time of coagulation with this method to range from 1.5 to 3 minutes, the average being a little lower than Wright's figures, 2.5 minutes. It may not be amiss to say that this seemingly simple method requires not a little practice if constant results are to be obtained.

The results of the observations are presented in the tables on pages 380-1.

X in case Brown indicates the beginning of the treatment, XX indicates the withdrawal of the calcium, XXX its resumption. X in case Griffith indicates beginning of treatment; XX indicates its rigorous enforcement, since the urine gave evidence of neglect of orders.

The average output of urine of Brown under voluntary conditions was always over 1100 cc.; under the Tufnell regimen it averaged 440 cc. Griffith's urine naturally fluctuated from 950 to 1300 cc.; under the treatment the average daily output was 525 cc. Prior to beginning treatment both patients were upon the same diet and drinking water, yet Brown eliminated by the urine only about 0.200 grm. $Ca_3(PO_4)_2$, while Griffith eliminated over 0.750 grm., a further illustration of the wide individual variations in urinary calcium elimi-

nation so often noted by different observers. In the case of Brown, despite the dry diet, the administration of calcium increased markedly the urinary elimination of that element, often to twice or thrice its natural quantity. Upon withdrawal of the drug (at XX) the quantity in the urine fell at once, and again rose promptly when its administration was resumed (at XXX). In the case of Griffith, the administration of calcium without strict enforcement of the dry diet (from X to XX) caused a considerable increase in its urinary elimination. But when the drinking was rigidly reduced the urinary elimination of calcium fell below the quantities previously eliminated while upon a normal diet without treatment. Even thus, however, the quantities eliminated were greater than in the case of Brown. It seems clear from these figures and from what is well known regarding the effect of the free use of water upon the absorption and urinary elimination of calcium salts, that were it held of prime importance to saturate the body with these salts, abundance and not restriction of water would be indicated.

That the ingestion of calcium affects the quantity in the circulating blood is suggested but not demonstrated by my analyses. Before the treatment, in the case of Brown, the quantities were 0.0172 and 0.0158 grm., during the treatment 0.022 and 0.0179 $Ca_3(PO_4)_2$ in 100 cc. blood; in the case of Griffith, before treatment, 0.018 and 0.0191 grm., during treatment 0.0146 and 0.015 grm., thus inclining to agree with the urinary elimination. It is clear, however, that so many other factors are concerned in this question that no conclusion can be established on this point.

The specific gravity of the blood in both cases was not materially affected by the treatment—1.0534, 1.051 before treatment, and 1.053, 1.052 during treatment, in the case of Brown; 1.060, 1.0607 before treatment, and 1.0598, 1.0609 during treatment, in that of Griffith. During the course of the treatment in both cases the number of red cells and the percentage of hæmoglobin diminished somewhat. Since the estimation of the specific gravity of the blood by weighing is much more accurate than the clinical methods of Thoma and Fleischl, I am compelled to look upon the apparent reductions either as

erroneous or as due to peripheral conditions. Both the constancy of the specific gravity and the reductions in the results of the clinical blood examinations show that in these cases no diminution in the water of the blood was effected by the treatment.

The same result is clearly shown by the percentages of the volume of plasma in the volume of native blood. These were in the case of Brown, 61.75 and 64 per cent before treatment, and 66 and 64 per cent during treatment; in the case of Griffith, 57.4 and 56.8 per cent before treatment, and 57 and 57.2 per cent during treatment. Any oligohydræmia would result in a diminution of the volume-percentage of the plasma, and since in the case of Brown the variations were in the direction of hydræmia and in the case of Griffith the results were constant, it is again clear that in these cases no oligohydræmia was effected by the treatment.

The plasma-nitrogen, plasma-albumin (factor 6.38) and quantities of albumin in the plasma of 100 cc. of blood were quite constant in the case of Brown, except for the second estimation, in which all were noticeably low. The patient at that time was suffering from an attack of influenza, but since that was before the beginning of the treatment, and since the two later determinations under treatment agreed with the earlier one prior to treatment, the absence of changes is clearly shown. In the case of Griffith the results were constant, except in the third estimation, in which they were high. The causes of this are obscure, since the percentage-volume of the plasma and the specific gravity were not altered. That the nitrogen and albumin of the plasma should vary constantly with the volume of plasma and the specific gravity of the blood is, however, not to be expected, since so many other factors are concerned in the latter.

The fibrin-nitrogen was not increased by the treatment beyond the range of error of the method. The only notable variation was the reduction observed at the time of the second estimation of Brown (before treatment), 0.0593, when suffering from the attack of influenza already mentioned. It is, therefore, clear that the treatment in these two cases did not increase the quantity of fibrin in the native volume of blood. The gross phenomena of clotting were in all cases

closely observed in the blood drawn apart for the estimation of calcium; in no case was the clot larger or firmer, nor did it retract or contract more quickly than normal.*

The time of coagulation as determined by Wright's method varied in the two cases. In Brown it was noticeably reduced during treatment below the figures noted previous to treatment, and reduced beyond the range of error of the method. During the six days when the calcium was withdrawn no differences in the time of clotting could be noted. In the case of Griffith, although there were marked variations both up and down, in general the time of clotting was not shortened. I believe, therefore, that in these two cases no acceleration in the process of clotting was effected by the use of calcium, since in Griffith, who best absorbed his calcium, no changes were noted, while in Brown the acceleration persisted while the drug was withheld. No inferences can be drawn respecting the influence of the Tufnell treatment upon the time of coagulation, since the results in the two cases do not agree.

The action of the calcium salts upon coagulation is and must remain purely empirical until the chemical relations of the process are better understood. Having apparently demolished the older views, Lilienfeld and his followers have in turn had grave doubts thrown upon their own by Pekelharing, Hammarsten and others,† and only thus much appears definitely known: that under certain circumstances, under the influence of several ill-understood factors, blood coagulates, and the product fibrin contains calcium and a proteid, plus how much more we know not, in a molecule of undetermined quantity. Under such circumstances discussion of the relations of calcium salts to blood coagulation under pathological circumstances would be profitless.

* Realizing that terms of nitrogen do not convey to the general reader a clearly defined idea of quantity, I have converted the figures of nitrogen into terms of fibrin in order to make the results more tangible, using as a basis of calculation the formula of Schmiedeberg; this formula, however, can be accepted only in a provisional sense. The quantities of fibrin thus determined read as follows: Brown, before treatment, 0.464 and 0.355; during treatment, 0.433 and 0.475 grm.; Griffith, before treatment, 0.457 and 0.450; during treatment, 0.478 and 0.466 grm.

† For example, Hammarsten, *Zeitschr. f. phys. Chem.* xxii, 333, and Cramer, ibidem, xxiii, 74.

While it seems clear that in every point respecting the *modus operandi* of this mixed treatment for aneurism and its influence upon the blood the result was entirely negative, I wish most definitely to state that under the treatment both patients improved. Brown received but slight betterment, the dilatation seemed simply held in check. Griffith, however, improved markedly; the tumor and the physical signs and symptoms receded progressively until he was finally discharged with no symptoms and no signs except dulness beneath the manubrium and a faint bruit. Brown was kept constantly in bed. Griffith was allowed the liberty of the hospital and its grounds, so that in his improvement rest played no role.

TABLE I.—CASE, BROWN.

X indicates beginning of treatment; XX, withdrawal of calcium; XXX, its resumption.

	Quantity of urine in cc. per diem.	Urinary calcium $Ca_3(PO_4)_2$ in grm. per diem.	Blood calcium $Ca_3(PO_4)_2$ in grm. in 100 cc.	Sp. gr. of blood.	Percentage volume of plasma.	Plasma-nitrogen in 100 cc. plasma.	Plasma-albumin in 100 cc.	Albumin in plasma of 100 cc. blood.	Fibrin-nitrogen in 100 cc. blood.	Coagulation time in minutes and seconds.	Venesection.	Number of red blood corpuscles and percentage of hæmoglobin.*
	1040	0.1915	....	....	....	....	....	....	....	3.10	....	4820000, 85%
	1180	0.2164	0.0172	1.0534	61.75%	1.322 grm.	8.432 grm.	5.207 grm.	0.0776 grm.	2.50	300 cc.	4780000, 87
X	1070	0.2218	0.0158	1.051	64	1.165	7.433	4.757	0.0593	2.30	260	4620000, 83
	320	0.571	....	....	....	....	....	....	....	2.30	....	4750000, 80
	500	0.540	....	....	....	....	....	....	....	1.55	....	4620000, 80
	445	0.504	....	....	....	....	....	....	....	2.15	....	4780000, 85
	520	0.432	...	....	....	....	....	....	....	2.09	....	4720000, 80
	425	0.399	....	....	....	....	....	....	....	2.04	....	4760000, 82
	480	0.756	0.022	1.053	66	1.223	7.803	5.150	0.0725	2.18	250	4690000, 85
	425	0.416	....	....	....	....	....	....	....	2.25	....	4610000, 80
XX	420	0.246	....	....	....	....	....	....	....	2.01	....	4480000, 80
	380	0.185	....	....	....	....	....	....	....	2.09	....	4480000, 78
XXX	490	0.241	....	....	....	....	....	....	....	2.11	....	4580000, 75
	430	0.364	....	....	....	....	....	....	....	2.18	....	4520000, 78
	410	0.296	0.179	1.052	64	1.271	8.109	5.190	0.0795	2.05	270	4450000, 75

*Last two figures hæmoglobin %. Thoma-Zeiss and Fleischl apparatus.

TABLE II.—CASE, GRIFFITH.

X indicates beginning of treatment; XX, its rigorous enforcement.

	Quantity of urine in cc. per diem.	Urinary calcium $Ca_3(PO_4)_2$ in grm. per diem.	Blood calcium $Ca_3(PO_4)_2$ in grm. in 100 cc.	Sp. gr. of blood.	Percentage volume of plasma.	Plasma-nitrogen in 100 cc.	Plasma-albumin in 100 cc.	Albumin in plasma of 100 cc. blood.	Fibrin-nitrogen in 100 cc. blood.	Coagulation time in minutes and seconds.	Venesection.	Number of red blood corpuscles and percentage of hæmoglobin.*
	1160	0.860	....	....	....	....	....	....	....	2.15	....	5050000, 90%
	1240	0.889	0.018	1.060	57.4%	1.276 grm.	8.141 grm.	4.673 grm.	0.0765 grm.	2.20	260 cc.	4940000, 88
	1195	0.784	0.0191	1.0607	56.8	1.291	8.237	4.679	0.0752	1.55	250	4960000, 95
X	980	1.134	....	....	....	....	....	....	....	2.30	....	4910000, 88
	975	1.088	....	....	....	....	....	....	....	1.40	....	4930000, 85
	1210	0.972	....	....	....	....	....	....	....	2.02	....	4990000, 85
XX	580	0.652	....	....	....	....	....	....	....	1.59	....	4860000, 88
	550	0.720	....	....	....	....	....	....	....	2.10	....	4920000, 88
	560	0.380	0.0146	1.0598	57	1.351	8.619	4.913	0.080	2.08	225	4890000, 90
	525	0.535	....	....	....	....	....	....	....	2.08	....	4810000, 85
	400	0.498	....	....	....	....	....	....	....	2.12	....	4850000, 80
	625	0.671	....	....	....	....	....	....	....	2.50	....	4750000, 85
	490	0.574	....	....	....	....	....	....	....	1.59	....	4880000, 88
	460	0.560	0.015	1.0609	57.2	1.265	8.071	4.617	0.078	2.10	240	4830000, 85

* Last two figures hæmoglobin %. Thoma-Zeiss and Fleischl apparatus.

EXPERIMENTS ON THE APPLICATION OF THE ROENTGEN RAYS TO THE STUDY OF ANATOMY.*

By ERNEST AMORY CODMAN, M. D., Boston.

PLATES XXIX-XXXVI.

The field of gross anatomy has been so well covered in the last century, and the dissecting knife and the method of frozen sections have enabled the anatomist to see so much of the internal structure of the body, that little has been left in this direction to the province of the Roentgen ray.

This does not apply, however, to pathological conditions of the bone in the living, for there the X-ray can do what the knife and frozen section cannot. For this reason it is important to have a knowledge of the normal X-ray anatomy of the bones, for the X-ray picture differs both from the photograph of the bare bones and from the frozen section, as it shows outline, internal structure and differences in density. The series † of skiagraphs which I have taken of the normal joints have their chief value from this point of view, as they show what may be considered the normal X-ray anatomy.

The skiagraphs accompanying this article were all taken from the cadaver (most of them from the same subject) by using a focus tube stimulated with a seven-inch spark induction coil. The current was about fifteen amperes, with a pressure of four volts in the primary coil. The break used was between copper surfaces immersed in distilled water.

In looking at skiagraphs there are several distortions and inaccuracies which must be borne in mind. One essential for a good skiagraph is an accurately focussed tube. The cathode rays are focussed

* This paper is the report by the author to the Committee on the Bullard Fellowship of the Harvard Medical School, May 1, 1897.

† Referring to album presented with this report to the Committee on the Bullard Fellowship and now in the Warren Museum.

by a concave aluminum electrode on the centre of a disc of platinum or the anode. The point where the stream of cathode rays strikes is the point from which the X-rays are generated. That this point should be as small as possible is important, in order that no penumbra should be formed. I have been fortunate in this series in having a tube in which this point was small, hence the outlines are sharply defined.

We may consider, then, that the X-rays proceed from practically a single point. In looking at a skiagraph we must consider it as a complete whole, made up of the shadows of the different parts of the object. The size and shape of the shadow of each part will depend on the relations of the distances between the point of light, the part of the object, and the plate. Thus the distance between the light and the plate remaining the same, the farther an object is from the plate the larger will be its shadow; and the farther an object, or part of an object, from the base of the perpendicular from the point of light to the plane of the plate the more will its shadow be distorted, the distortion being due to elongation in the direction of a line from the base of the perpendicular through the object.

When the object is at the same time away from the plate and away from the base of the perpendicular, both of these distortions will take place. Further, since each object or part of an object contains only a certain amount of shadow-casting material, the farther it is from the plate the greater will be the size and the less the density of the shadow.

An important corollary follows from this. A picture of a less dense object may be taken through a more dense, *e. g.* the ear through the skull. This can be done when the densities of the two objects are not greatly contrasted; but when one object is so dense as to be practically impenetrable to the rays, its shadow will of course obliterate the shadows of anything either in front of or behind it. This is generally true of metallic objects.

Another source of inaccuracy of these pictures is the fact that the rays in penetrating large objects are more or less diffused. This diffusion tends to blur the outlines of the parts of the object at a

distance from the plate. It may also assist in making more distinct the objects in direct contact with the plate; for instance, in the picture of the ear taken through the skull, the diffused rays from the other parts of the head may help to take the picture of the ear, because they blur all those parts of the plate which are not protected by the ear, which comes in close contact with the wrapper.

It will be seen from the foregoing that in order to reduce these inaccuracies to a minimum the distance from the plate to the tube should be as great as possible, and that from the object to the plate as short as possible. The distance, therefore, must be gauged by the intensity of the rays and convenient time of exposure. We must also remember that the wider the object, or the deeper the object, the greater must be the distance between the plate and the tube. For instance, the skiagraph of a hand at ten inches is very little distorted, while that of two hands side by side would be considerably distorted, because the rays would strike the outer fingers at more acute angles.

A knee at ten inches, though little wider than a hand, would yet be considerably distorted, for it is so thick that the parts furthest from the plate would be much enlarged; the external condyle of the femur (the knee being on the inner side, for instance) would appear much larger than the internal (Fig. 1).

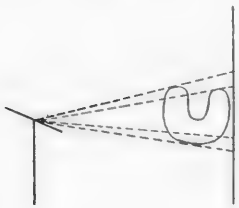

Fig. 1.

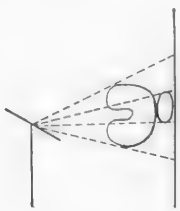

Fig. 2.

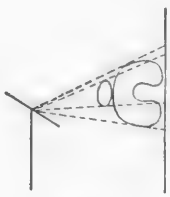

Fig. 3.

In order to reduce these distortions to a minimum the tube should be put as far away as is possible with a convenient time of exposure.

On the other hand, however, advantage can be taken of this distortion in taking a photograph of the patella. If the plate is put to the posterior side of the knee (Fig. 3) the shadow of the patella will

be hardly, if at all, distinguishable. This would be true also if the plate were on the anterior side and the light far away, for the shadows of the femur would be dense enough to overshadow that of the patella. If, however, the plate be on the anterior side and the tube very near the posterior (Fig. 2) the shadow of the femur will be broadened and diminished in density, so that the shadow of the patella, which is but little magnified and as dense as before, will be seen through it.

The use of the fluoroscope is a practical way of determining the intensity of the rays. In determining the distance we must remember that the intensity of the light varies inversely as the square of the distance. In determining the time we can calculate that what we lose by doubling the distance we gain by multiplying the time of exposure by four.

THE STUDY OF THE MECHANISM OF THE JOINTS.

This has been undertaken in two ways. First, by skiagraphing the normal joints in their forced extreme positions, extreme flexion, adduction, etc. Secondly, by watching the movements with the fluoroscope. As the parts of the object near the plate show best, it has been necessary to take each position from both sides.

One thing which will at once arrest attention is the great distance that apparently intervenes between the bones. This is in part due to the fact that the articular cartilages, being easily traversed by the rays, do not cast a shadow. The accompanying skiagraphs of hands (Plates XXIX-XXXIV) were exposed at a distance of about twelve inches for 3 to 5 minutes.

The wrist joint has proved most interesting in this study, and the points brought out will be found in the following description:

For convenience we may consider the wrist joint to be made up of four immobile and two mobile elements.

I. Immobile (*i. e.* those made up of single bones or of a group of bones, the components of which cannot change relative positions). These are:

1. Metacarpal of thumb.
2. Metacarpal of ring finger.

3. Metacarpal of little finger.

4. Metacarpal of index and middle finger with trapezium, trapezoid, os magnum, and unciform.

This last group are so firmly attached to one another that they move as a whole, practically as one bone. No doubt, however, their ligamentous attachments allow of more or less spring in strained positions of the hand brought about by external force.

II. Mobile (the components of which change relative positions).

1. The intermediate row of carpal bones composed of scaphoid, semilunar, cuneiform and pisiform.

2. Radius and ulna.

From the skiagraphs it is found that the carpus and metacarpus are, in any of the extreme positions, in practically the same relation to the radius, no matter what the relation of the radius to the ulna, whether pronation or supination. This is due to the more or less flexible fibro-cartilage, which in any position completes the cup of the radial joint. The question of mechanism, then, is further simplified by leaving out the ulna, which really does not enter into the construction of the joint except as a pivot. The pisiform also does not enter the mechanism, serving only as a sesamoid for the ulnar tendons.

Proceeding to eliminate other accessory elements, we can disregard the metacarpals of the thumb, ring and index fingers, each of which moves independently on the large fixed element composed of the os magnum, etc. The thumb forms a typical saddle joint with the trapezium, with the pommels of the saddle so low that motion is allowed in a small circle, either as rotation within the circumference or straight motions on any of the radii. The metacarpal of the ring finger is allowed a slight antero-posterior motion of a few degrees. That of the little finger the same of slightly greater extent, with possibly a degree of adduction. This leaves us with:

I. The large compound fixed element of os magnum, etc.

II. The radius with fibro-cartilage.

III. The intermediate element of scaphoid, semilunar and cuneiform.

These constitute the real wrist joint, and their movements are very beautiful (Figs. 5 and 6).

The joint may be compared to a ball-bearing with the balls irregular and allowing only limited motions. It must be remembered, too, that there is no real separation of the component parts, for besides the ligaments tending to bind them together there is the atmospheric pressure which closes down the capsules and ligaments as each bone is drawn along another.

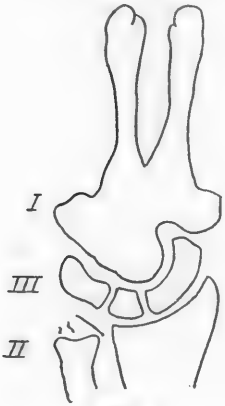

Fig. 4.

Fig. 5.

Fig. 6.

Starting from the straight position in antero-posterior view we put the joint in adduction (cf. Plates XXIX and XXX). The radius remaining fixed, the mobile intermediate row may move as a whole on the radius. This does happen to the extent seen in the skiagraphs. In the meanwhile the os magnum, etc., move on the intermediate row. This motion would be very slight in extent, since the outer side of the head of the os magnum comes against the lower end of the scaphoid, were it not for the fact that the mobile row is straightened out both by the pressure of the uneiform on the cuneiform and of the magnum on the scaphoid (Plate XXX). The lower end of the scaphoid is also pulled down by the trapezium, so that it is extended. In fact, all the intermediate row in adduction are extended and in abduction

flexed. This Dr. Dwight has beautifully shown by driving a long pin into each bone and watching the ends of the pins while manipulating the joint.

The os magnum element is also slightly flexed in adduction and extended in abduction to compensate for the opposite movements in the intermediate row.

In the same way the movements of flexion and extension are almost equally divided between the radio-carpal and intercarpal joint (Figs. 5 and 6 and Plates XXXII, XXXIII, XXXIV). The semilunar may be taken as the type of the intercarpal row and will be observed in the radiographs to tilt through an angle of about 90°. In like manner the os magnum tilts on it as it moves through an angle of 90°, giving to the whole joint its range of nearly 180° between the extreme positions.

This comparatively simple view of this beautiful mechanism is, I think, an accurate one, and the points are well confirmed by the anatomical specimens.

The chief value of the radiographs of the other joints is in forming a series for comparison with pathological conditions. The necessity for training in interpreting skiagraphs is evident from the foregoing description of the distortions of these pictures, so that an idea of the normal X-ray anatomy is necessary for an intelligent interpretation of abnormal or diseased conditions of the bones. The skiagraphs of the trochlear surface of the elbow joints form a good illustration of this, as it requires considerable study of the skiagraphs of the elbow taken from the side to enable one to make out the position of the bones.

The skiagraphs of the bones of the foot did not prove as interesting as I had expected, for the relative position of the bones of the tarsus remains almost the same in plantar as in dorsal flexion. Most of the motion takes place between the astragalus and tibia, while there is a slight general motion between the other bones which is pretty equally divided.

Many of these skiagraphs are doubly interesting, as they were taken from cadavers which had been injected with the usual mixture of

starch and a red mercuric salt for coloring. The mercuric salt (red oxide) being more or less opaque to the rays, renders the arteries visible. The study of the arteries in this way has much the same advantages as those given by corrosions for the general grouping, regional distribution and relation to the bones (Plate XXXV). The smaller branches can be seen in a way impossible in dissection.

Other skiagraphs were also taken with metallic mercury injected into the joint capsules. Metallic mercury was found difficult to handle, expensive, and not capable of an even distribution in the cavities on account of its own cohesive tendency. A saturated solution of plumbic nitrate was therefore used instead and was found cheaper and more satisfactory.

I have spoken of the distortion of Roentgen-ray pictures, and particularly the fact that the parts of an object at a distance from the plate have their shadows magnified. Curiously enough this distortion can be very nearly corrected by the use of the stereoscope. Two skiagraphs may be taken of an object which, when placed in the stereoscope, will give one picture of that object which will have the appearance of solidity (Plate XXXVI). To take stereoscopic pictures two exposures are made with the plate and object in exactly the same relation to each other, but with the light in a slightly altered position in relation to both. This should be done by moving the tube to the right or left a distance corresponding to the normal space between the eyes or a little less, after taking the first skiagraph in the usual position. At the same time a new plate is substituted for the old one, care being taken to put it in exactly the same place.

In the stereoscopic pictures the skiagraphs were reduced to small "transparencies" and printed so as to make the bones and arteries appear white for greater effect. With a given pair of these stereoscopic pictures by transposing the prints in the stereoscope, *i. e.* placing them as (1-2) or (2-1), we can look at the object as if from behind or from before. For instance, in such skiagraphs as those of a hand we seem to look at either the palmar view or the dorsal, that is, the palmar arch will appear in front or behind the bone according as the one or the other of the prints occupies the right-hand space.

Now since the parts of the object farthest from the plate are enlarged, the best arrangement will be that which in the stereoscope will make us seem to look at the enlarged or near side of the object. The enlargement will then be that which would naturally exist on the retina in looking at the object from the side away from the plate. Thus the distortion is in part corrected. The criticism of these pictures, however, may justly be made that the parts of the object which show the clearest and with most detail are those which appear farthest off in the stereoscope. This of course is contrary to the simple optical effect. It is, however, not noticeable except in stereoscopic pictures of deep objects.

DESCRIPTION OF PLATES XXIX–XXXVI.

PLATE XXIX.

Wrist in straight position. Notice relation of scaphoid and semilunar to radius and compare Plates XXX and XXXI.

PLATE XXX.

Wrist in adduction. Notice leverage of head of os magnum on scaphoid and fulcrum between cuneiform and unciform. By this leverage the curve of the intermediate row is straightened out and becomes the arc of a larger circle. Cf. relations in Plate XXXI.

PLATE XXXI.

Wrist in abduction. Scaphoid is flexed and hence seems shorter than in Plate XXX, os magnum approaches scaphoid and the intermediate row becomes arc of a smaller circle.

PLATES XXXII, XXXIII AND XXXIV.

Wrist in straight position (Plate XXXII), extension (Plate XXXIII), and flexion (Plate XXXIV), as seen from side. The os magnum and semilunar show more clearly than the other bones, and may be taken as types of the two rows. By comparison of the three figures the division of the motion between the radio-carpal and intercarpal joint will be observed.

PLATE XXXV.

Adult male pelvis with arterial injection. There is a sound in the bladder and a scalpel under the left side of the cadaver. Reduced from 18 x 24 in.

PLATE XXXVI.

Stereoscopic views of hand with arterial injection. To obtain the solid effect one must look at the pictures cross-eyed, or with a stereoscope, having cut them out and transposed them.

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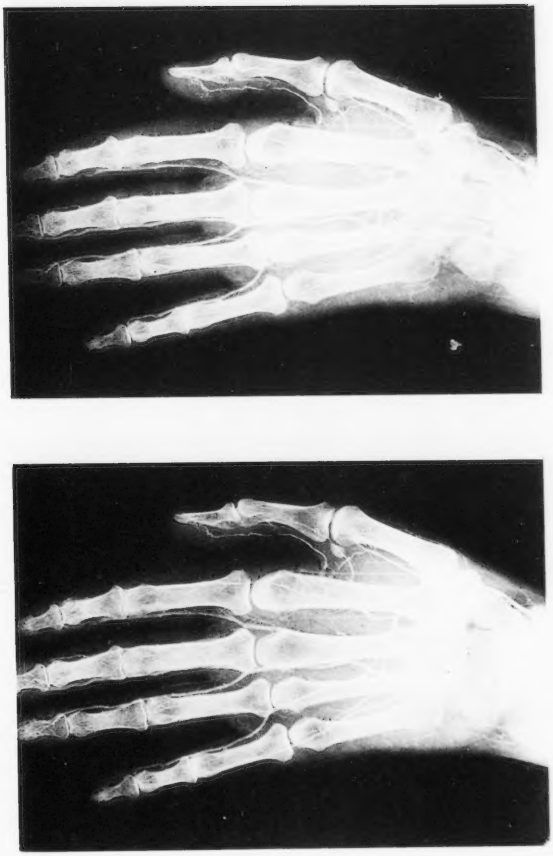

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